09/510562

Exhibit A

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U.S. Food and Drug Administration

INTERCENTER AGREEMENT BETWEEN THE CENTER FOR **DRUG** EVALUATION AND RESEARCH AND THE CENTER FOR **BIOLOGICS** EVALUATION AND RESEARCH

This document outlines a working agreement between the Center for Biologics Evaluation and Research (CBER) and the Center for Drug Evaluation and Research (CDER). It assigns to the Centers jurisdiction for regulation of Drug and Biological products and combinations of Drugs and Biological products, and describes those product characteristics or medical indications that will require a collaborative review effort by the two Centers. The document also contains agreements on mechanisms for dispute resolution, and on the logistic aspects of collaborative reviews, including the use of advisory committees.

This agreement arose from the need to allocate responsibility for regulation of various new products, some of which do not easily fit into the traditional jurisdictional responsibilities of the two Centers, and from the desire to use available resources and expertise efficiently. This document is intended to provide a clear basis for the Centers' program planning and for guidance to applicants. Submissions should be made directly to the lead Center identified in this document.

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I. EFFECTIVE DATE

This agreement takes effect on October 31, 1991.

II. GENERAL DESCRIPTION

CDER is the lead Center for regulation of human drugs that are regulated by FDA under the authority of the Federal Food Drug and Cosmetic (FD&C) Act. Drug products are defined in section 201(g) of the FD&C Act.

CBER is the lead center for regulating biological products. Biological products are regulated under authority of, and defined in, Section 35l of the Public Health Services (PHS) Act and in 21 CFR 600.3. Products that are biological products are also either drugs or medical devices as defined in the FD&C Act.

This agreement supersedes all prior agreements.

III. CENTER JURISDICTION OVER PRODUCT CLASSES.

Under this agreement, Center jurisdiction over products is allocated by product class. A product class is defined as a distinct category of agents recognizable by physical characteristics, source materials or pharmacologic properties. Examples of product classes include antibiotics, vaccines, hormones, and human blood derivatives.

In certain cases, for scientific or administrative reasons, the designated lead Center, with the concurrence of the

other Center, may transfer the responsibility for a specific product to the other Center. Such transferred products will ordinarily be regulated by the same mechanism (NDA or PLA) as the other products in the class to which they belong. This agreement continues the previous agreement under which CBER has had full responsibility for regulation of certain products used in conjunction with blood banking that are regulated as drugs under the authority of the FD&C Act.

Assignment of a product to a Center means that the Center is responsible for administering regulation of the product and for evaluation of product quality, i.e., manufacture, quality control and specifications. In certain cases (see Section IV, Medical Review Assignments), a major part of the pharmacological/toxicological, biopharmaceutic, and medical review may be conducted by the Center that is not assigned responsibility for the product. It is understood that assignment of product responsibility to one Center does not preclude consultation with the other Center on product-related issues. In all cases, the Center with product responsibility will have final responsibility for actions taken on the application.

Product Responsibility Assignments:

A. CDER is responsible for the following classes of drug and antibiotic products:

1. Naturally-occurring substances purified from mineral or plant source materials (excluding vaccines or allergenics);
2. products that are produced from non-human animal or solid human tissue sources (excluding animal-derived procoagulant products or antisera, venoms, red blood cell replacement products, vaccines, allergenic products, products composed of living cells, and certain other products listed under B. below)
3. antibiotics as defined by Section 507(a) of the FD&C Act, regardless of the method of manufacture:
4. certain agreed-upon classes of substances constitutively produced by fungi or bacteria including:
   a. disaccharidase inhibitors
   b. HMG-CoA reductase inhibitors;
5. chemically-synthesized molecules (excluding vaccines and allergenics) including:
   a. products produced by chemical synthesis that are intended to be analogies of cytokines, thrombolytics, or other biologic, or that function by binding to the receptors for biological products,
   b. chemically-synthesized mononucleotide or polynucleotide products, including products complementary to RNA or DNA sequences; and
6. hormone products, regardless of method of manufacturing, e.g., insulin, human growth hormone, pituitary hormones.

B. CBER is responsible for the following classes of products:

1. Biological products subject to licensure:
   a. vaccines, regardless of method of manufacture including those vaccines which at the effective date of this agreement are being studied under active INDs administered by CDER (For the purpose of this agreement, a vaccine is defined as an agent administered for the purpose of eliciting an antigen-specific cellular or humoral immune response);
   b. in vivo diagnostic allergenic products, in vivo diagnostic tests for DTH, and allergens regardless of the method of manufacture intended for therapeutic use as "hyposensitization" agents;
   c. human blood or human blood-derived products including placental blood-derived products, animal-derived procoagulant products and animal or cell culture-derived hemoglobin-based products intended to act as red blood cell substitutes;
   d. immunoglobulin products, whether monoclonal or polyclonal, produced in humans, animals or in cell culture;
   e. products composed of or intended to contain intact cells or intact microorganisms including bacteria, fungi, viruses or virus pseudotypes, or viral vectors;
   f. protein, peptide or carbohydrate products produced by cell culture excepting antibiotics, hormones, products listed in A.3. above, and products previously derived from human or animal tissue and regulated as approved drugs;
   g. protein products produced in animal body fluids by genetic alteration of the animal, i.e., transgenic animals; and
   h. animal venoms or constituents of venoms.
2. Other product classes:
   a. Synthetically-produced allergenic products that are intended to specifically alter the immune response to a specific antigen or allergen; and
   b. certain drugs used in conjunction with blood banking and/or transfusion.

C. Exceptions:

1. All products that are subject to approved or pending NDAs or PLAs as of the effective date of this agreement will be left under that regulatory mechanism and under the jurisdiction of the center that currently administers the NDA or PLA.
2. New products that use the same active ingredient(s) as the above approved products will be assigned to the same lead Center and regulated by the same mechanism (PLA or NDA) as the approved products. Questions about the similarity of active ingredients will be settled by the CBER-CDER jurisdiction committee.

D. Combination products:

Products that are combinations of one or more drug and one or more biologic products will be assigned based on the products primary mode of action. This mechanism assigns administration and precinct quality responsibility: the medical, pharmacological and other reviews will be conducted as described in Sections IV. and V. below.

1. Assigned to CBER:
   a. Combination products that consist of a biological product from a product class subject to licensure (including biological products that have been chemically modified) combined with a radioactive component.
   b. Combination products that consist of a biological product component used as a mode of localization and a toxin component that is not itself a drug product (e.g., ricin A toxin) used as an effector.
   c. Combination products that consist of drug component and a biological component where the drug product enhances the efficacy or ameliorates the toxicity of the biological product.
2. Assigned to CDER:
   a. Combination products that consist of a biological product component used as a mode of localization or used to affect the distribution of the product, combined with a nonradioactive drug component used as an effector.
   b. Combination products that consist of a biological component and a drug component where the biological component enhances the efficacy or ameliorates the toxicity of the drug product.

IV. MEDICAL REVIEW ASSIGNMENTS

This agreement provides for apportioning medical review between the Centers, based on existing programs and foci of clinical expertise. This arrangement will facilitate efficient utilization of existing resources and rational program planning. CBER has program areas and medical expertise predominantly in the fields of allergy, clinical immunology and rheumatology, infectious diseases, hematology, and coagulation and oncology. CDER has programs in a wider variety of clinical areas, including a number of those where CBER also has ongoing programs.

This agreement outlines three categories of medical indications. The first group includes clinical indications for which CBER will ordinarily take the lead in medical review. For these areas, CBER agrees to maintain programs and medical expertise. The second group outlines the areas for which CDER will ordinarily take the lead in medical review and will maintain programs and expertise. A third category is those medical areas for which both Centers expect to maintain programs and expertise. Products with clinical indications in this category will ordinarily have the medical review performed by the same Center assigned responsibility for product quality review and application administration.

The assignment of review responsibility to one Center does not preclude review involvement of the other Center. In those clinical indications for which clinical review may occur in either Center, the Centers agree to ensure consistency in standards for clinical evaluation and outcome measures by cooperating in the development of pertinent policy, guidelines and regulations and by continuing to hold working meetings at the staff level to discuss clinical issues. Periodic intercenter coordination meetings on products intended for treatment of AIDS, and cancer will continue and will include specific planning of the coordinated review of pending applications.

Medical and other reviewers from a second Center who are participating in a collaborative review will be expected to fully participate in the review processes of the Center with responsibility for administration of the application (see Section VI, Collaborative Review Process). It is understood that the primary Center will have final signatory authority on all actions; however the collaborating reviewers are expected to develop specific recommendations based on their review of the data, on their interaction with other experts reviewing the application, and on input from their supervisors.

In certain cases (e.g. allergy and dermatology, or clinical immunology and GI), the clinical indication may be a

hybrid between several areas. In these cases a medical review team consisting of appropriate experts from both Centers may be formed.

Either Center may decline to participate in a requested collaborative review if expertise or resources are not available.

The following are the medical review responsibilities:

A. Clinical data which will ordinarily be reviewed by CBER medical staff.

Products intended for or acting by a mechanism of:

1. replacing the 0_2carrying function of red blood cells;
2. directly and specifically activating the proliferation of hematopoietic cells;
3. replacing plasma coagulation factors;
4. achieving a passive immune response;
5. diagnosing allergy or delayed type hypersensitivity by in vivo testing; and
6. inducing an antigen-specific active immune response or tolerant state (i.e., vaccines and allergenics) but excepting vaccines intended to induce a contraceptive state.

B. Clinical data which will ordinarily be reviewed by CDER medical staff.

Products intended for diagnostic or therapeutic use in:

1. radiotherapy,
2. hormonal disorders,
3. obstetrics, gynecology and contraception,
4. metabolic disorders,
5. dermatological disorders,
6. ophthalmological disorders.
7. neurological or psychiatric disorders,
8. surgical indications including use in anesthesia,
9. dental practice,
10. analgesia,
11. pulmonary disorders,
12. gastrointestinal disorders,
13. cardiovascular disorders, and
14. renal disorders.

C. Clinical data which may be appropriately reviewed by medical staff in either Center, in accordance with Center product jurisdiction assignment.

Products intended for:

1. radioimaging,
2. immunosuppression,
3. diagnosis, prevention or treatment of infectious diseases,
4. diagnosis, prevention or treatment of neoplastic disorders,
5. diagnosis, prevention or treatment of rheumatological disorders,
6. diagnosis, prevention or treatment of immunodeficiency disorders,
7. diagnosis, prevention or treatment of hematological disorders other than those mentioned in A.), or
8. nonantigen-specific diagnosis, prevention or treatment of allergic disorders.

V. RESPONSIBILITY FOR OTHER REVIEWS

A. Pharmacology/Toxicology Review

1. When one Center is performing medical review of a product assigned to the other, responsibility for the pharmacology and toxicological review will ordinarily be shared between the two Centers. Prior to initiating a PLA or NDA review, a formal agreement delineating responsibilities for pharmacology/toxicology review will be made.
2. The Centers will ensure consistency in standards for pharmacological/toxicological product evaluation by

continuing to cooperate in the development of pertinent policy, guidelines and regulations and by continuing to hold working meetings at the staff level to discuss issues.

B. Additional Aspects of Product Quality Review

1. The Center assigned responsibility for product quality is responsible for obtaining or performing any necessary facility inspections, reviews of such inspectional findings, and product test results.
2. Where relevant expertise in product quality aspects is not available in the Center with product jurisdiction and is present in the other Center, collaborative review will be sought and provided.

In cases where a product regulated by CDER is derived from human source material, including cell culture, it is agreed that CDER will request and CBER will provide consultation on testing for adventitious agents and related issues. To provide consistency, in cases involving animal source material, each Center agrees to consult with the other when issues refuted to policy development arise.

VI. COLLABORATIVE REVIEW PROCESS

Collaborative review is defined as a review process where the responsibility for medical review is assigned to a different Center than the responsibility for regulatory administrative actions and review of product quality. Collaborative review is distinct from consultation in that consultants provide opinions to primary reviewers whereas collaborative reviewers are responsible for developing a definitive review in a particular area. Such a review is a permanent part of the administrative record. It is nonetheless subject to appropriate supervisory review from the official delegated responsibility for final agency action on an application.

A. IND Review.

When an IND is filed in CDER for a clinical use requiring CBER review input, the CDER Division Director will send a request specifying the review assistance sought, accompanied by one copy of the application to the Director, DBIND, CBER, with informational copies of the request to the Director of the relevant CDER Office and the Director, CBER. The Director, DBIND, will, according to CBER procedures, obtain any necessary input from other CBER divisions.

Similarly, when an IND is filed in CBER for a clinical use for which CDER has review responsibility, the Director, DBIND, CBER will send a request for clinical review and one copy of the IND to the relevant CDER Division with an informational copy of the request to the Director of the relevant CDER office.

Reviewers from the collaborating Center will be expected to participate fully in all aspects of review during the IND process. The organizational component with IND responsibility for administrative and product quality review; i.e., either DBIND or the CDER NDE review Division, will be responsible for coordination of document flow, arranging all meetings and correspondence with the sponsor.

B. NDA/PLA Review.

1. Requests for intercenter clinical review will be submitted from the Center with administrative responsibility for the application, to the office of the other Center Director.
2. Each Center will have in place administrative procedures for formal assessment of review staff to the NDA or PLA with concurrence in the assignment of the individuals' immediate supervisor(s) and Division Director.
3. Each Center will develop short written procedures regarding the contribution required for intercenter or collaborative review, including the types of reviews required and the appropriate extent of documentation. Where CDER has administrative responsibility, reviews by CBER staff will be documented in writing and returned through the appropriate division and office director, for incorporation into the administrative record. Where CBER has administrative responsibility, reviews by CDER staff will be written full reviews through the division (and, if applicable, office) director. The reviewer will meet with the PLA committee and will coordinate the review through that committee with the product quality review in CBER.
4. A designated supervisor, usually the CBER PLA committee chair or CDER Division Director, with responsibility for ensuring the review of the application in the responsible Center, will negotiate an agreement with the supervisor(s) of the collaborating reviewer(s) with regard to the contribution (i.e., time and work product) expected and also will provide ongoing feedback, as required, with regard to timeliness, setting of priorities, and quality of work. At each designated performance review date, and after completion of an action on the application, this designated supervisor will be responsible for submitting a written evaluation of the collaborating reviewer(s) to their permanent supervisor.

5. The Center with administrative responsibility will be responsible for final decision making. It is the responsibility of collaborating reviewers to form and document their best scientific and regulatory assessment and recommendations regarding assigned reviews. It is the responsibility of the delegated official in the Center with administrative responsibility to take final action regarding the application. In the case of differences in judgment, when the designated official in the center with administrative responsibility intends to override the opinion of the collaborating reviewer(s), there shall be a memorandum to the administrative record documenting the reasons for overriding the opinion of the clinical reviewer, which will be copied to and discussed with the collaborating reviewers and the collaborating reviewers' Center Director (or delegate) before final action is taken.

C. Advisory Committee Review

It is recognized that many of the new products subject to collaborative review will generate complex scientific and medical regulatory issues and that the Centers will need to utilize all available advice during the regulatory decision-making process. Therefore, every attempt will be made to involve pertinent advisory committees from either or both Centers for products under collaborative review.

VII. INTERCENTER JURISDICTIONAL COMMITTEE

An intercenter jurisdictional committee is now formed. It is composed of one representative and one alternate from each Center. The committee members will meet on an ad hoc basis, will discuss all jurisdictional questions, and will be expected to handle the majority of requests for jurisdiction assignments. Where this committee cannot agree upon jurisdictional assignment, or where the sponsor requests review of assignment at the agency level, the product jurisdiction procedures will be used.

Signed:

Carl C. Peck, M.D.
Director
Center for Drug Evaluation and Research
Date: 10/25/91

Signed:

Gerald V. Quinnan, Jr., M.D.
Acting Director
Center for Biologics Evaluation and Research
Date: 10/25/91

Concur:

David A. Kessler, M.D.
Commissioner of Food and Drugs

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A Brief History of the Center f r Drug Evaluation and Research

The Center for Drug Evaluation and Research (CDER), which assures that safe and effective drugs are available to the American people, has gone through a functional and organizational metamorphosis since it began as a one-man operation to assess significant drug problems in the marketplace on the eve of the 1906 Pure Food and Drugs Act. In part, this change reflects the evolution of drug law and the chemotherapeutic revolution over the 20th century--and the concomitant changes in responsibilities of the Food and Drug Administration. But the change also reflects external and internal decisions on how best to provide safe and effective drugs to patients. Every branch of government, as well as other interests affected by FDA's policies, have had a role in the way this agency regulates drugs. The following story summarizes some of the dynamics involved in the history of the drug regulatory function at FDA, especially with respect to the many organizational upheavals this function experienced over the past 90 years.

During the 1902 annual meeting of the American Pharmaceutical Association, Harvey Wiley, the Chief Chemist of the Bureau of Chemistry, announced the formation of a Drug Laboratory within his organization. Wiley intended the Laboratory to assist with standardizing pharmaceuticals and unifying analytical results. One of the nominees to lead this function was Lyman Frederic Kebler, the Chief Chemist at Smith Kline and French and a recognized expert in the detection of drug adulteration. Appointed Director of the Drug Laboratory in November 1902, he assumed the duties in March 1903.

Initially, the Drug Laboratory worked on a variety of projects. One of the first was an investigation of the reagents used by the Bureau, which Kebler soon learned were not completely pure. The Laboratory spent much of its time in search of methods to improve pharmaceutical analyses. Kebler also alerted the public to problems with the drug supply in general.

Three years later, behind the long-time lobbying of Wiley, the Pure Food and Drugs Act became law; this prohibited interstate commerce of mislabeled and adulterated drugs and food. Wiley himself emphasized food-related issues as a greater health concern, but he gave some attention to patent medicines and prescription drugs; his successor, Carl Alsberg, elevated the importance of drug matters. By 1908 the Drug Laboratory underwent its first of what would be many major reorganizations. Renamed the Drug Division, it was divided into four laboratories: the Drug Inspection Laboratory, directed by George Hoover; the Synthetic Products Laboratory which W.O. Emery headed; the Essential Oils Laboratory, under E.K. Nelson; and the Pharmacological Laboratory, directed by William Salant. Kebler remained the Division Director.

The main focus of the Essential Oils Laboratory was the analysis of oils used therapeutically either alone or in combination with other chemicals, such as root-beer extract and oil of wintergreen. The Synthetic Products Laboratory was responsible for examinations of synthetic remedies, including the popular headache mixtures, and active ingredients in crude drug products. The Pharmacological Laboratory investigated the physiological effects of drugs on animals. Most of their early efforts centered on caffeine, a subject of great interest to Wiley. Finally, the Drug Inspection Laboratory was the principal enforcement arm of the Division. For example, from 1909-1910 this Laboratory scrutinized over 900 domestic drug samples, around 1000 imported drugs, and recommended prosecution of 115 samples.

One of the first major challenges to drug regulation under the 1906 Act came in 1910. The Bureau had seized a large quantity of "Johnson's Mild Combination Treatment for Cancer," a worthless product that bore false therapeutic claims on its label. When the case came to trial, the judge determined that claims made for effectiveness were not within the scope of the Pure Food and Drugs Act, and ruled against the government. In 1912, Congress issued corrective legislation. The Sherley Amendment brought therapeutic claims within the jurisdiction of the Pure Food and Drugs Act, but required the Bureau to prove those claims to be false **and** fraudulent before they would be judged as illegal.

By this time, the scope of the Drug Inspection Laboratory's work had grown. For example, they investigated methodologies for the determination of morphine, nitroglycerin, and other drugs in combination preparations. Also, the Laboratory collaborated with the U.S. Pharmacopeia (USP) in a study of drug standards. The work of the Division of Drugs was not limited to domestic drug problems. They also studied imported drugs and chemicals and imported products of dubious therapeutic value. The Division also spent considerable time on an investigation of contaminated chloroform. Several manufacturers had been distributing chloroform in tin containers, which was prone to decompose into a substandard product compared to USP chloroform stored in glass.

In the mid 1910s, the Division of Drugs added two new components. The Pharmacognosy Laboratory was created in 1914. In addition to investigating crude drug products, this Laboratory studied improvements in crude drug processing to reduce waste. In 1916 the Division established an office to investigate false and fraudulent labeling of drugs. This effort stemmed directly from the Sherley Amendment and was directed by M.W. Glover, a physician on detail from the U. S. Public Health Service (USPHS).

The Bureau created the independent Office of Drug Administration in the early 1920s, headed by Glover, to assist the Division of Drugs with issues specific to drug labeling. In March 1923 Glover was recalled to the USPHS and the Office was abolished. At the same time, Kebler became head of the autonomous Office of Special Collaborative Investigations, which worked on mail fraud issues with the Post Office Department, and the Division of Drugs was reorganized. In 1923, the Office of Drug Control replaced both the Office of Drug Administration and the Division of Drugs. Directed by George Hoover and organized in parallel with the new Office of Food Control, the Office was responsible for all work in the control of drugs, including crude drugs, manufactured drug ingredients, drug preparations, and patent medicines.

The Office continued to collaborate with outside concerns, including trade associations and the USP. The desire for increased manufacturing accuracy led to the formation of contact committees within the associations. After the committees completed studies on ways to improve accuracy, recommendations on implementation were published in the trade journals. In 1926 the Bureau's cooperation with the USP grew even greater through a program to provide standardized product samples of bioassayed drugs, such as digitalis, to be used as reference standards. This program continued until 1930.

After receiving reports of several deaths relating to impure anesthetics, the Office launched an investigation into the cause. There were several anesthetics on the market at the time, but this investigation focused on ether and ethylene. This was broadened over the years and continued into the mid-1930s. By the conclusion of the investigation, the Office determined that the decomposition of anesthetics was a result of poor manufacturing practices. As part of this inquiry, the Office established a Laboratory dedicated to drug analysis.

Beginning in 1928, the drug regulatory staff within the Food, Drug and Insecticide Administration (as the agency was known then) underwent several important personnel changes. George Hoover left the agency after directing the Office of Drug Control for five years and Lyman Kebler resigned as Director of Special Collaborative Investigations. As a result, Special Collaborative Investigations became a unit in the Office of Drug Control, which by this time also contained a Chemical Unit, a Medical Unit, a Veterinary Unit, and a Pharmacology Unit. The new Director of Drug Control was James J. Durrett, like Kebler a physician and pharmacist; Durrett was a professor of public health at the University of Tennessee at the time he joined FDIA.

A highlight in the Office's research was a study of ergot pharmacology conducted by Marvin R. Thompson. Initially, the Office had been concerned about decomposition of crude ergot; analysis had shown that this typically occurred during shipping. But the lack of knowledge of the pharmacology of ergot became obvious during this investigation. This led to the beginning of Thompson's work. In 1929 his paper was awarded the Ebert Prize of the American Pharmaceutical Association, bestowed annually for exceptional work in pharmacology. Thompson's paper was groundbreaking in many areas. He proposed modifications in the methods for assaying ergot, and showed that the USP standard for ergot

could be improved by changing the preparation techniques.

Shortcomings in the Pure Food and Drugs Act had been obvious since it became law. One weakness was the lack of authority to stop distribution of dangerous preparations claiming to reduce weight. In 1934 the Office of Drug Control, headed since 1931 by physician Frederick J. Cullen, began investigations on products containing dinitrophenol. This was a component in diet preparations that increased metabolic rate to dangerous levels, and it was responsible for many deaths and injuries. Since the law did not mandate drug safety, the Office of Drug Control could not seize the products, and was limited to posting warnings.

In 1935 James J. Durrett returned to the Food and Drug Administration to direct the drug regulatory function, a function that once again was named the Drug Division. That same year the pharmacology responsibilities, theretofore part of the Division, became a separate, independent office, headed by Erwin E. Nelson. Nelson held the unique position of consultant to the agency while he remained on the faculty at Michigan from 1919-1937 and chair of the Pharmacology department of Tulane from 1937-1943. The main reason for the separation was to accommodate the increasing pharmacology needs of the food industry, especially in investigations of the effects of poisons and impurities in foods.

Continuing problems with dangerous drugs that fell outside the parameters of the Pure Food and Drugs Act finally received national attention with the Elixir Sulfanilamide disaster in 1937. Massengill distributed this preparation without testing for safety (which was not required by law). Because it contained diethylene glycol as a vehicle, a chemical analogue of antifreeze, over 100 people died, many of whom were children. In June 1938 President Roosevelt signed the Federal Food, Drug and Cosmetic Act into law. Among other things, this law required new drugs to be tested for safety before marketing, the results of which would be submitted to FDA in a new drug application (NDA). The law also required that drugs have adequate labeling for safe use. All drug advertising was assigned to the Federal Trade Commission.

Three months after the President signed the 1938 Act, Theodore Klumpp, who received his medical training at Harvard and served on the faculty at Yale University, assumed leadership of the Drug Division after Durrett resigned. By this time, the Division consisted of a Chemical Section, a Collaboration Section, a Medical Section, and a Veterinary Section, and most of the work focused on reviewing NDAs. Within the first year of this requirement, the Division received over 1200 applications.

The early 1940s saw three major additions to FDA's responsibilities in the drugs area. The Insulin Amendment, passed in 1941, required all batches of insulin to be tested for purity, strength, quality, and identity before marketing. The testing was conducted by a unit in the Pharmacology Division. Also starting in 1941, the agency required prescriber labeling for all new drugs, in concert with the adequate directions for use provision of the 1938 Act. The Penicillin Amendment was passed in 1945, modeled on the Insulin Amendment. The former required batch certification of drugs wholly or partially composed of penicillin. Subsequent amendments extended the certification requirement to other antibiotics. Responsibility for the testing was placed in another separate and independent office, the Division of Penicillin Control and Immunology. Divided into four sections, Penicillin Certification, Immunology, Antiseptics, and Antibiotics, this Division's responsibility extended beyond testing of penicillin. In 1949 the Division was renamed the Division of Antibiotics to reflect the growing scope of functions and antibiotics.

The Drug Division at this time was under the leadership of a new director, Robert P. Herwick, who came to this position in 1941. He was trained in chemistry, pharmacology, toxicology, medicine, and law. Under Herwick's direction, the Division retained the same sections -- medical, chemical, and veterinary. The medical section was responsible for reviewing the safety and labeling of new drugs, and consulted on court cases. The veterinary section served the same function for animal drugs. The chemical section analyzed medicines and developed analytical methods for by field chemists.

By 1945 the Division Director also served as the Chief Medical Officer of FDA, and soon thereafter the Drug Division was renamed the Division of Medicine. Herwick resigned in

1947 and was succeeded by Robert Stormont. Stormont was trained in pharmacology and medicine, and had served in the Naval Medical Corps before joining the FDA in 1946.

Erwin E. Nelson, the pharmacology consultant who directed the New Drug Section beginning in 1947, was promoted to Medical Director after Stormont's departure. Ralph Smith became the Chief of the New Drug Section after Nelson moved up, a position he held until the mid-1960s. Both an M.D. and Ph.D., he came to the FDA from the Tulane University School of Medicine, where he served as chairman of the Pharmacology Department. Under Smith's leadership, the agency approved over 7,000 New Drug Applications. Soon after the ascendancy of Nelson and Smith, Congress passed another law with a significant impact on drug regulation. The Durham-Humphrey Amendment of 1951 clarified the vague line between prescription and nonprescription drugs theretofore under the law. The Amendment specifically stated that dangerous drugs, defined by several parameters, could not be dispensed without a prescription, witnessed by the prescription legend: "Caution: Federal law prohibits dispensing without prescription."

Drug regulation farther down in the distribution system came under scrutiny in 1955, when FDA undertook a pilot study of adverse drug reaction reporting. In cooperation with the American Society of Hospital Pharmacists, the American Medical Association, and others, the study focused on reactions reported by hospitals and pharmacists. Adverse reaction reporting at this time was voluntary and reports normally were scarce. This study blossomed into a more ambitious effort in 1957 to test a large-scale system for voluntary reporting to assist with post-marketing evaluation of new drugs. By 1963 the study had evolved into a voluntary reporting system with almost 200 hospitals participating.

The Secretary of Health, Education, and Welfare formed the Citizens Advisory Committee in 1955 to review practices of the FDA and make recommendations to improve resource utilization. The 14-member Committee, consisting of leaders of industry as well as consumers, was chaired by G. Cullen Thomas of General Mills. Their report of June 1955 contained over 100 recommendations, including over two dozen regarding drugs specifically. Most of the drug recommendations dealt with the NDA program, especially suggestions for accelerating the review program. In general, the Committee recommended that the staff at FDA be increased at least threefold, and the budget as much as fourfold.

As a result of the recommendations of the 1955 Citizens Advisory Committee, the Division of Medicine underwent a major reorganization and became the Bureau of Medicine in 1957. By this time the Bureau was under Albert Holland, formerly of the New York University College of Medicine and Armour Laboratories, where he had been medical director. Holland had been appointed Medical Director in March 1954, following the resignation of Nelson in 1952.

The agency-wide reorganization that led to the creation of the Bureau of Medicine also established the Bureau of Biological and Physical Sciences, formed by grouping the seven scientific Divisions in FDA. These Divisions, including Pharmacology and Antibiotics, were engaged in various projects, both food- and drug-related (e.g., development of methods to improve analysis and specifications for insulin and certifiable antibiotics.) In 1959 the Bureau began an internal publication, Bureau By-Lines, that fostered communication between the headquarters and field laboratories. Bureau By-Lines continued until 1982. At the end of the 1950s, the Bureau of Medicine consisted of five branches, the New Drug Branch, the Drug and Device Branch, the Veterinary Medicine Branch, the Medical Antibiotics Branch, and the Research and Reference Branch. The new medical director, William Kessenich, came to FDA in 1959 from the Department of Internal Medicine at Georgetown University.

Strengthening the drug provisions of the 1938 Act were the focus of Senate hearings held in June of 1960. These hearings, chaired by Senator Estes Kefauver of the Subcommittee on Antitrust and Monopoly of the Committee on the Judiciary, resulted in S.3815. This bill aimed to protect the public health by instituting certain manufacturing practices, expanding antibiotic certification to all antibiotics, and by other measures.

During the Kefauver hearings, FDA received an NDA for Kevadon, the brand of thalidomide

that the William Merrell Company hoped to market in the U.S. Despite ongoing pressure from the firm, medical officer Frances Kelsey refused to allow the NDA to become effective because of insufficient safety data. By 1962 thalidomide's horrifying effects on newborns became known. Even though Kevadon was never approved for marketing, Merrell had distributed over two million tablets for investigational use, use which the law and regulations left mostly unchecked. Once thalidomide's deleterious effects became known, the agency moved quickly to recover the supply from physicians, pharmacists, and patients. For her efforts, Kelsey received the President's Distinguished Federal Civilian Service Award in 1962, the highest civilian honor available to government employees.

As a result of the narrowly avoided tragedy from thalidomide, Senator Estes Kefauver re-introduced his bill. On October 10 President Kennedy signed the Drug Amendments of 1962, also known as the Kefauver-Harris Amendments. These Amendments required drug manufacturers to prove to the FDA that their products were both safe and effective prior to marketing. They also required that all antibiotics be certified, and gave FDA control over prescription drug advertising. With the new law, the review of antibiotic NDAs was transferred from the Division of New Drugs to the Division of Antibiotics. In May 1961, the designation "Division" replaced "Branch."

The Drug Amendments also addressed the use of drugs in clinical trials, including a requirement of informed consent by subjects. FDA had to be provided with full details of the clinical investigations, including drug distribution, and the clinical studies had to be based on previous animal investigations to assure safety. FDA formed the first drugs advisory committee, the Advisory Committee on Investigational New Drugs, for assistance in implementing the new law. The Committee, chaired by Walter Modell of Cornell University, served as a de facto interface between FDA and clinical investigators and other scientists around the country.

In the wake of the new law, the Division of New Drugs was restructured into five branches in 1962. The Investigational Drug Branch, directed by Kelsey, evaluated proposed clinical trials for compliance with investigational drug regulations. Earl Meyers, who began his career with FDA in 1939, was the director of the Controls Evaluation Branch, which reviewed the manufacturing controls proposed by drugs makers. The Medical Evaluation Branch assessed safety and efficacy data in NDAs. The New Drug Status Branch, under John Palmer, consulted with manufacturers about their NDAs and proposed dosing schedules for new products. The last branch, New Drug Surveillance, evaluated adverse reaction reports. Ralph Smith remained Division director, though he also served as acting Medical Director from the time Kessenich departed (1962) until March 1964, when Joseph Sadusk was appointed to that position.

Sadusk chaired the Department of Preventive Medicine and Community Health at George Washington University before he joined FDA. Under Sadusk, the Bureau of Medicine consisted of four Divisions: Medical Review, directed by Howard Weinstein, New Drugs, directed by Smith, Research and Reference, under George Saiger, and Veterinary Medical, which was headed by Charles Durbin.

The Bureau of Scientific Standards and Evaluation and the Bureau of Scientific Research also had drug responsibilities. These Bureaus replaced the Bureau of Biological and Physical Sciences in 1964. The Bureau of Scientific Research was in charge of long-term scientific projects, under the direction of Daniel Banes; the Division of Pharmacology and the Division of Pharmaceutical Chemistry were located here. The Scientific Standards Bureau, led by Robert Roe, was responsible for decisions on certification and petitions; included in this Bureau was the Division of Antibiotics and Insulin Certification. In 1964 responsibility for antibiotics was shifted back to the Division of New Drugs in the Bureau of Medicine to centralize the review of NDAs for all types of human drugs.

Uncertainty about the safety of America's drug supply continued after the passage of the Kefauver-Harris Amendments. As a result, Congress opened hearings in March 1964, chaired by Representative L.H. Fountain, to investigate FDA's efforts to promote drug safety. But Fountain's hearings took a comprehensive look at the agency's regulation of drugs, especially those that were removed from the market.

To further comply with the drug amendments of 1962 the FDA contracted in 1966 with the National Academy of Sciences/National Research Council to study drugs approved between 1938 and 1962 from the standpoint of efficacy. The Drug Efficacy Study Implementation (DESI) evaluated over 3000 separate products and over 16,000 therapeutic claims. The last NAS/NRC report was submitted in 1969, but the contract was extended through 1973 to cover ongoing issues. The initial agency review of the NAS/NRC reports by the task force was completed in November 1970. One of the early effects of the DESI study was the development of the Abbreviated New Drug Application (ANDA). ANDAs were accepted for reviewed products that required changes in existing labeling to be in compliance. In September 1981 final regulatory action had been taken on 90% of all DESI products. By 1984, final action had been completed on 3,443 products; of these, 2,225 were found to be effective, 1,051 were found not effective, and 167 were pending.

In May 1972, FDA applied the principle of a retrospective review to over-the-counter (OTC) drugs. The structure for this OTC review would necessarily be different than that of the prescription drug review, mainly because of the vast array of available OTC products -- hundreds of thousands of different preparations. The OTC review focused on active ingredients, around 1,000 different items, and panels of experts were convened to evaluate these drugs. The agency would publish the results as a series of monographs in the Code of Federal Regulations, specifying the active ingredients, restrictions on formulations, and labeling by therapeutic category.

FDA formed seventeen panels, consisting of seven voting members (medical, dental, and scientific experts) and non-voting representatives for industry and consumers. The panels were responsible for arranging the drugs into three categories: safe and effective, unsafe and/or ineffective (which should no longer be marketed), probably safe and effective, but needing further testing to establish significant proof. The review is ongoing. The agency eventually decided that drugs in the last category, like those in the second, would be taken off the market until sufficient proof dictated otherwise.

The Bureau of Medicine's interest in increasing efficiency for clearing new drugs and distributing work among staff members led to yet another restructuring in 1965. The new Bureau consisted of five Divisions and the Office of the Medical Director (still Sadusk). The five Divisions were New Drugs, (Smith); Medical Review, (Howard Weinstein); Medical Information, formerly the Division of Research and Reference, (Donald Levitt); Veterinary Medicine, (Charles Durbin); and Antibiotic Drugs, (Raymond Barzilai). The Division of Antibiotic Drugs continued to evaluate antibiotic NDAs, but certification of antibiotics remained in the Bureau of Scientific Standards and Evaluation. The Medical Advisory Board also was established at this time to advise FDA on the problems faced by the industry, the medical community, and other health-related areas. Sadusk chaired the Board, and its members included leaders in medicine, pharmacology, dentistry, and veterinary medicine from across the country.

The following year, 1966, the Bureau of Medicine introduced an Office and Division structure. The Office of New Drugs was responsible for reviewing all aspects of NDAs and investigational new drugs. The Office of Drug Surveillance reviewed adverse drug reaction reports and supplemental drug applications. Finally, the Office of Medical Review was responsible for regulatory actions. This latest reorganization also reflected the establishment of the Bureau of Veterinary Medicine in November 1965. Directed by M. Robert Clarkson, the Bureau was responsible for review of both veterinary drugs and devices. The human device program remained in the Bureau of Medicine until 1971. Sadusk resigned after overseeing the reorganization. Herbert Ley, who had been at both Harvard Medical School and George Washington University, succeeded Sadusk in September 1966. In 1967 the Bureau of Medicine replaced the Office of Drug Surveillance with the Office of Marketed Drugs, which was responsible for approval of supplemental applications. In addition, the Bureau established the Office of Medical Support to centralize a variety of functions in the Bureau, such as medical advertising and adverse reaction reporting.

In December 1969, a Departmental study known as the Malek Report recommended a major reorganization of FDA along products lines. Indeed the Bureaus of Compliance, Medicine,

and Science soon were replaced by the Bureau of Drugs and the Bureau of Foods, Pesticides and Product Safety. To form the Bureau of Drugs, the drug and device activities of the Bureau of Medicine were combined with the pharmaceutical science responsibilities of the Bureau of Science and the drug and device compliance activities from the Associate Commissioner for Science. The new Bureau of Drugs consisted of four Offices: New Drugs, Marketed Drugs, Compliance, and Pharmaceutical Sciences.

The National Center for Drug Analysis (NCDA) opened in St. Louis, Missouri, in July 1967 to conduct large scale tests of drug products. Prior to this, NCDA was part of the Division of Pharmaceutical Sciences in the Bureau of Science (formed in 1966 after the Bureau of Scientific Standards and Evaluation merged with the Bureau of Scientific Research). In its first year, the NCDA examined over 7,000 samples. From 1973 until 1981, the Bureau was under the direction of J. Richard Crout. Crout, a pharmacologist at Michigan State, came to the attention of Henry Simmons (1970-1973), Crout's predecessor, while serving on the Ad Hoc Science Advisory Committee (Ritts Committee). The latter investigated the place of science within FDA. Following a 1973 management study of the overall drug function, the Bureau of Drugs reorganized in November 1974 into seven offices: Planning and Evaluation, Compliance, Information Systems, Biometrics and Epidemiology, Pharmaceutical Research and Testing, Drug Monographs, and New Drug Evaluation.

During the early 1970s, the FDA started two new forums to increase drug communication with the public. The Bureau of Drugs launched the FDA Drug Bulletin in 1971. The Bulletin alerted physicians and pharmacists to changes in drug use and labeling requirements. The National Drug Experience Reporting System also began in 1971. The NAS had been studying the problem of not only how to catalogue and store information about adverse drug reactions, drug abuse, and drug interactions, but also how that information could be made available to health professionals. The study concluded that since FDA had already collected the data, they should take the lead on creating and maintaining the system.

A renewed push for changes in drug regulation began at the highest level of the Department. HEW Secretary Joseph Califano felt that, for such changes to be effective, they had to be made through legislation rather than administrative policy. The initial bill, introduced in Congress on March 17, 1978, was titled the Drug Regulation Reform. It contained nine main provisions: to increase consumer protection, encourage drug innovation, increase consumer information, protect patient rights, improve FDA enforcement, promote competition and cost savings through generic drugs, increase FDA's public accountability, make additional drugs available, and encourage research and training. The effort during 1978 was unsuccessful, but the bill was reintroduced the following year. The Senate approved the bill in September 1979, but the House did not take action and the measure died.

However, efforts to promote one of the provisions in the Senate-approved version of the Drug Regulation Reform bill, the requirement for drug manufacturers to provide package inserts for all prescription products, continued after the reform bill. Since 1970 the FDA had required the inserts only for oral contraceptives. In July 1979 FDA proposed a program to provide patients with additional information about their prescription drugs, including a description of the drug's uses, risks, and side effects. Under the proposal, the manufacturer would print the information and the provider (pharmacist, doctor, nurse, etc.) would give the insert to the patient. But by September 1980, under the weight of well-organized opposition to the program, FDA dropped the insert project.

During the early 1980s the Bureau of Drugs underwent several substantial organizational changes, ranging from discrete changes in branch substructures to a revamping of the entire organization. Among the less ambitious alterations, the Bureau restructured the Division of Drug Information Resources in the fall of 1980, and the Prescription Drug Labeling Staff was transferred from the Office of the Division Director to the Division of Drug Advertising the following year. In March 1982 several Divisions changed names (and, to varying extents, responsibilities): the Division of Product Quality became the Division of Drug Quality Evaluation, the Division of Drug Manufacturing changed to the Division of Drug Quality Compliance, and the Division of Drug Advertising became the Division of Drug Advertising and Labeling.

The biggest organizational change during this time was the merger of the Bureau of Drugs and the Bureau of Biologics to form the National Center for Drugs and Biologics (NCDB). The new Director of the NCDB, Harry Meyer, Jr., had been head of the Bureau of Biologics. The purpose of this reorganization was to streamline FDA's approval procedures in drugs and biologics and to increase the public's assurance of the safety and effectiveness of the drug supply.

The NCDB consisted of five offices: New Drug Evaluation, Drugs, Biologics, Management, and Scientific Advisors and Consultants. The Office of New Drug Evaluation, directed by Robert Temple, was formed from the six Divisions in the Bureau of Drugs that reviewed NDAs. Jerome Halperin was the first Director of the Office of Drugs, which included the remaining Divisions from the Bureau of Drugs that conducted research and developed standards for the safety and effectiveness of drugs. The Office of Biologics, under John Petricciani, managed the Divisions from the Bureau of Biologics. Administrative functions were in the Office of Management, which Russell Abbott headed. The establishment of the Office of Scientific Advisors and Consultants, directed by Morris Schaeffer, facilitated scientific proficiency and research in drugs and biologics. Finally, the reorganization abolished the National Center for Antibiotics Analysis. Formed in 1968 from the old Division of Antibiotics and Insulin, the Center had been responsible for certification and testing of antibiotics. In 1981 the FDA proposed to phase out the certification program by late 1982, and the program ended on October 1, 1982.

In 1984 all of the National Centers within FDA were redesignated simply as centers. At this time, the Center for Drugs and Biologics established or reengineered five offices. The Office of Compliance, directed by Daniel Michels, was made up of the Divisions of Drug Labeling Compliance, Drug Quality Compliance, Drug Quality Evaluation, Scientific Investigations, Biological Product Compliance, and Regulatory Affairs. The Office of Management, still under Abbott, was comprised of the Divisions of Planning and Evaluation, Administrative Management, and Drug Information Resources. Temple became Director of the Office of Drug Research and Review and led the Divisions of Cardio-Renal Drug Products, Neuropharmacological Drug Products, Oncology and Radiopharmaceutical Drug Products, Surgical-Dental Drug Products, Drug Biology, Drug Chemistry, and Drug Analysis. Peter Rheinstein headed the Office of Drug Standards, which consisted of the Divisions of OTC Drug Evaluation, Biopharmaceutics, Generic Drugs, and Drug Advertising and Labeling. The Office of Epidemiology and Biostatistics, under Gerald Faich, was made up of the Division of Biometrics and the Division of Drug Experience. Finally, Elaine Esber led the Office of Biologics Research and Review.

Drug responsibilities increased in several ways in the mid-1980s. The Orphan Drug Act of 1983 employed several means to promote development of products for rare diseases. Among the provisions of this law, the sponsors of drug candidates could petition the agency for assistance in planning animal and clinical protocols. Also, the sponsor was allowed seven years of marketing protection for unpatentable products, and the law provided a 50 percent tax credit for investigation expenses. As a result of this Act, in early 1983 an Orphan Product Development office was established in the Office of the Commissioner, under Marion Finkel. Also in 1983, Congress passed the Federal Anti-Tampering Act in the wake of the Tylenol poisonings. This law amended the U.S. Code to provide penalties for tampering with or threatening to tamper with any product covered by the Food, Drug, and Cosmetic Act.

Advertising in professional journals was a well-accepted practice, where doctors, pharmacists and other health professionals could read information on side effects and other disclosure data adjacent to the promotional information. Direct to consumer television advertising of prescription drugs emerged in May 1983. Boots Pharmaceuticals was the first manufacturer to use this new venue in promoting its Rufen brand of ibuprofen. FDA took action against the advertisement out of concern that consumers would not be able to read the long list of side effects that flashed quickly across the screen. The commercial was replaced with an acceptable version.

The Drug Price Competition and Patent Term Restoration Act of 1984 gave FDA the authority to accept abbreviated new drug applications (ANDAs) for generic versions of post-1962 drugs. The first approved ANDA was for generic disopyramide, marketed as

Norpace and used in the treatment of cardiac arrhythmias. As a result of this legislation, several Divisions in the Office of Drug Standards added branches to assist with the review of ANDAs. The law also provided manufacturers with the opportunity to apply for an extra five years of patent protection to make up for time lost during the FDA approval process.

Several important organizational changes emerged during this period. In 1985, the Drugs and Biologics Fraud Branch was established within the Division of Drug Labeling Compliance to combat health fraud in the drug and biologic areas. That same year, the Division of Drug Chemistry was abolished and its staff reassigned to the Division of Drug Analysis for NDA review. Also, the Division of Drug and Biologic Product Experience was renamed the Division of Epidemiology and Surveillance.

The structure of the Office of Compliance changed in 1986, resulting in five Divisions: Drug Labeling Compliance, Drug Quality Evaluation, Scientific Investigations, Regulatory Affairs, and Manufacturing and Product Quality. The Office of Consumer and Professional Affairs, formed after the merger of the Bureau of Drugs and the Bureau of Biologics, was abolished in 1987. Also in 1987, Paul Parkman became the Director of the Center for Drugs and Biologics, and Gerald Meyer became his deputy.

Additional laws and policies of the 1980s had an impact on drug approval and distribution. For example, the agency strengthened reporting requirements for adverse reactions in 1985. The new requirements addressed all prescription drugs, including older pharmaceuticals that predated FDA approval. New regulations for investigational drug development also went into effect in 1985. The new rules increased the availability of experimental drugs, including compassionate use of drugs under research for patients with serious and/or life-threatening conditions. In 1988 FDA promulgated treatment IND regulations. These allowed desperately ill patients to receive promising new drugs before full approval had been completed. Congress passed the Prescription Drug Marketing Act in the same year. This law prohibited the purchase, sale, trade, and -- with exceptions -- reimportation of drug samples. It also required drug wholesalers to register with states.

On October 6, 1987, the Center for Drugs and Biologics was split into the Center for Drug Evaluation and Research and the Center for Biologics Evaluation and Research (CBER). This split was necessary because of the increasing volume of NDAs, to provide proper high-level attention to the growing problem of AIDS, and to address other issues in drug and biologic evaluation. Carl Peck became the first Director of CDER, and Parkman was named Director of CBER. Peck, who had been Director of the Department of Clinical Pharmacology at the Uniformed Services University of the Health Sciences when he came to FDA, continued as Director until 1994, when Janet Woodcock became the second Director of CDER. Woodcock, whose background is in internal medicine and rheumatology, had been in CBER before assuming her position in the Center for Drugs.

When CDER began it consisted of six offices: Management (directed by Robert Bell), Compliance (Michels), Drug Standards (Rheinstein), Drug Evaluation I (Temple), Drug Evaluation II (James Bilstad), Epidemiology and Biostatistics (Faich), and Research Resources (Jerome Skelly). The Division of Antiviral Products was established in 1988 within the Office of Drug Evaluation II to assist with review of drugs for AIDS and other indications. The Office of the Center Director added two new staff offices in 1989. The Professional Development Staff developed and coordinated programs to assist with recruiting and training of Center staff, and the Pilot Drug Evaluation Staff fostered new ideas to streamline the drug approval process.

Also at this time, CDER established the Office of Generic Drugs to assume responsibility for review of ANDAs, which had been located in the Office of Drug Standards. In addition, the Generic Drugs Advisory Committee was formed to assist the Office of Generic Drugs with approval issues. This Committee advised the Office on scientific and technical matters related to the safety and effectiveness of generic drugs. In the wake of the convictions of five FDA reviewers for unlawful contacts with regulated industry, Congress passed the Generic Drug Enforcement Act in 1992. This law provided a variety of penalties for illegal acts involved with ANDA approvals.

Reorganizations in response to scientific and legislative mandates continued in the 1990s. For example, the Office of Research Resources created the Division of Clinical Pharmacology. This Division, consisting of the Chemotherapy and Analytical Methodology Branch and the Preclinical Development Branch, studied clinical pharmacology and expanded CDER's interests in clinical investigations.

The Prescription Drug User Fee Act, passed in 1992, required drug and biologic manufacturers to pay fees to the FDA for the evaluation of NDAs and supplements. Also, the firms would pay an annual establishment fee and product fees. Congress required FDA to apply user fees to hire more reviewers, and thus expedite the reviews. Following the passage of this legislation, the number of new drug approvals has indeed increased steadily each year, from 63 in 1991 to a record 131 in 1996. Also, the number of approved new molecular entities (NMEs) increased from 30 in 1991 to 53 in 1996.

Recently CDER underwent a Center-wide reorganization, beginning in 1995. Within the Office of Drug Evaluation I (ODE I) the Division of Oncology and Pulmonary Drug Products was split into separate Divisions; the Division of Oncology Drug Products stayed in ODE I and the Division of Pulmonary Drug Products moved into ODE II. Also, nine new Offices were established and the functions of one were moved. Included in the new Offices were three additional Offices of Drug Evaluation, an Office of Training and Communication, the Office of Review Management, the Office of Pharmaceutical Science, the Office of New Drug Chemistry, the Office of Clinical Pharmacology and Biopharmaceutics, and the Office of Testing and Research. The functions of the Office of Over-the-Counter Drug Evaluation were transferred to ODE V.

Through the years, responsibilities within FDA for drug regulation have undergone major changes. Most of these came as a result of innovations in drug development and additions to legislative authority. When Lyman Kebler was hired in 1902, he was basically a one-man bureau who had corrupt reagents and half a desk to fight the most egregious offenses of a largely unregulated industry. As of 1994, CDER was the largest headquarters component of FDA, consisting of almost 1500 men and women working in several buildings. The complexity and challenges of drug review are multiplying as the sophistication of drug design and manufacturing increases, which speaks to the importance of maintaining a well-trained and adequately supported group of agency drug officials, for the good of the public health.

**Prepared by Donna Hamilton, Historian, FDA History Office, November 1997

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Exhibit B

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Autologous bone marrow transplantation (ABMT) for acute leukaemia in complete remission: a pilot study of 33 cases

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SUMMARY Thirty-three leukaemic patients in CR were treated by high-dose therapy followed by ABMT: 18 of them had acute non-lymphoblastic leukaemia (ANLL) in first remission (CR1) with a mean age of 23·7 years (3–44). All but one of them were conditioned with a polychemotherapy regimen including 6-thioguanine, Ara-C, CCNU, and cyclophosphamide. The marrow cells were purged by chemical means in 16 cases.

Five transplant-related deaths were observed: three cardiac failures, one interstitial pneumonitis and one aspergillus pneumonia. At the time of analysis (October 1984), four patients had relapsed and eight were still in unmaintained CR1 (44+, 46+, 30+, and five between 2·5+ and 8+ months post transplant).

Fifteen patients had acute lymphoblastic leukaemia: four were autografted in CR1 and 11 children were grafted in CR2; the conditioning regimen was fractionated total body irradiation followed by cyclophosphamide for all but one patient who was conditioned with BACT (Burkitt leukaemia); the marrow was purged by a chemical agent in 11 patients and by monoclonal antibodies and C′ in four: four out of 15 patients relapsed (two grafted in CR1 and two grafted in CR2); 10 patients are still in unmaintained CR: two adults grafted in CR1 (26+; 12+ months) and eight children with a mean follow-up of 13·4 months postgraft (2+–45+ months).

The clinical study leads to the following conclusions: in adult patients the

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marrow should be harvested during CR1 and at the time of minimal residual disease. The quality of previous chemotherapy and conditioning regimen prior to ABMT play a prominent role in the *in vivo* eradication of the leukaemic cells.

The real impact of marrow purging is still unknown and a larger series of homogeneous patients, conditioned with the same protocols and the same transplant timing, is required before any conclusions can be drawn.

High-dose cytoreductive therapy, followed by autologous bone marrow transplantation (ABMT), has been proposed as an alternative to conventional therapy for acute leukaemia in complete remission (Gorin *et al*, 1981; Izzi *et al*, 1983; Stewart *et al*, 1983; Burnett *et al*, 1984; Herve *et al*, 1984; Löwenberg *et al*, 1984; Santos & Kaizer, 1984; Velle Koop *et al*, 1984; Kaizer *et al*, 1985). Different protocols containing high-dose chemotherapy with or without total body irradiation (TBI) have been proposed. This investigational approach must be considered as an early massive consolidation, as in allogeneic bone marrow transplantation.

In a previous study (Herve *et al*, 1982) the feasibility of intensive therapy followed by ABMT in 11 acute non-lymphoblastic leukaemia (ANLL) cases in relapse was evaluated. Ten complete remissions (CR) with a mean CR duration of 9·7 months (range 4·5–15) were obtained without maintenance therapy. In most cases of ABMT in relapse the duration of remission appears to be short, with very few long-term survivors (Dicke *et al*, 1979; Gorin *et al*, 1981; Zander *et al*, 1981; Herve *et al*, 1983; Maraninchi *et al*, 1983; Dicke *et al*, 1984). At the time of relapse, massive therapy followed ABMT remains one of the most effective treatments to achieve CR. A second course of treatment (with a second marrow rescue) given after obtention of remission, may induce long-term CR (Anderson *et al*, 1984; Maraninchi *et al*, 1984) without maintenance therapy. In a second study, with the experience of allogeneic results (Thomas *et al*, 1983), high-dose cytoreductive therapy and ABMT were included in our protocol as an early intensive therapy following consolidation treatment of the first or second CR. The marrow rescue thus obtained at the time of marrow harvest may have the lowest level of residual clonogenic leukaemic cells.

Since 1981, *ex-vivo* purging of marrow prior to freezing has been carried out with a view to eliminating residual malignant cells. Nevertheless, it is very difficult to detect and quantify minimal residual disease in a remission marrow with the techniques available today.

Physical, immunological and pharmacological methods of purging remission bone marrows are available: physical fractionation of bone marrow cell suspensions by discontinuous albumin gradients has been extensively studied by Dicke *et al* (1979, 1984) without conclusive results. *Ex-vivo* purging with stable active cyclophosphamide derivatives (4HC or Asta-Z-7557) is widely used (Santos & Kaizer, 1984; Herve *et al*, 1984; Gorin, 1984) and other chemical agents are under investigation such as VP 16-213, a semisynthetic derivative of podophyllotoxin (Stiff *et al*, 1984; Kalwinsky *et al*, 1983). Polyclonal heteroantisera (Netzel *et al*, 1980) have been supplanted by monoclonal antibodies (MoAbs): mainly anti-CALLA MoAbs (Ritz *et al*, 1982) and anti-T cells MoAbs which are cytotoxic when used with rabbit complement (C').

Other immunological means of purging such as immunotoxins against T leukaemic cells

(Myers *et al*, 1984; Kersey *et al*, 1984) and immunomagnetic methods (Poynton *et al*, 1983) seem promising. All purging methods described previously could be rendered ineffective if, prior to ABMT, malignant cells have not been eliminated *in-vivo* by an effective high-dose chemoradiotherapy.

High-dose therapy followed by ABMT was performed on 33 leukaemic patients in CR between December 1980 and October 1984; 29 out of 33 patients received purged marrow: 24 with a pharmacological agent and five with MoAbs and rabbit C′.

PATIENTS AND METHODS

Patients

Thirty-three patients received high-dose combination therapy followed by ABMT: 18 were ANLL in first CR (CR1) and 15 were ALL in CR: four in CR1, 11 in CR2.

(a) *ANLL.* 18 patients (11 male, seven female) were treated in CR1: 13 adults and five children with a mean age of 23·7 years (range 3–44).

The 18 cases had the following subtypes of acute leukaemia according to the French–American–British (FAB) classification (Bennett *et al*, 1976): M_1 (five), M_2 (five), M_3 (two), M_4 (two), M_5 (three), M_6 (one patient). 16 patients received marrow purged by a pharmacological agent.

(b) *ALL.* Four ALL patients (two male, two female) were treated in CR1: three adults and one child. According to the FAB classification (Bennett *et al*, 1976) their subtypes were as follows: L_2 (three), L_3 (one) and their immunological phenotypes: T cell ALL (two cases), B cell ALL (one case) and one non-T non-B ALL (CALLA+).

11 children (10 male, one female), with a mean age of 8 years (range 2–14) were autografted in CR2. Nine of them had medullary relapse, two had extramedullary relapse (testicular and meningeal), five out of 11 were off therapy at the time of relapse.

According to the FAB classification their subtypes are as follows: L_1 in seven patients and L_2 in four patients, with the following immunological phenotypes: non-T non-B ALL in six cases (four CALLA+) and T-cell leukaemia in one case.

In three patients with high-risk features the bone marrow was harvested in CR1. The mean duration of the CR1 was 35·7 months (range 5–82) for the eight cases grafted in CR2. Fourteen patients received marrow purged either by a pharmacological agent (10 patients) or by anti-CALLA MoAbs (four patients).

Methods

(a) *Processing of bone marrow* (Fig1). Our methods have already been described (Herve *et al*, 1983). Marrow cells were collected under general anaesthesia by multiple aspirations from the sternum and iliac crests. The volume aspirated at each puncture site was limited to 3 ml in order to reduce dilution with peripheral blood. The collected cells were suspended in ACD-A and the marrow mononuclear cells were isolated using an IBM-2991 cell washer (Gilmore *et*

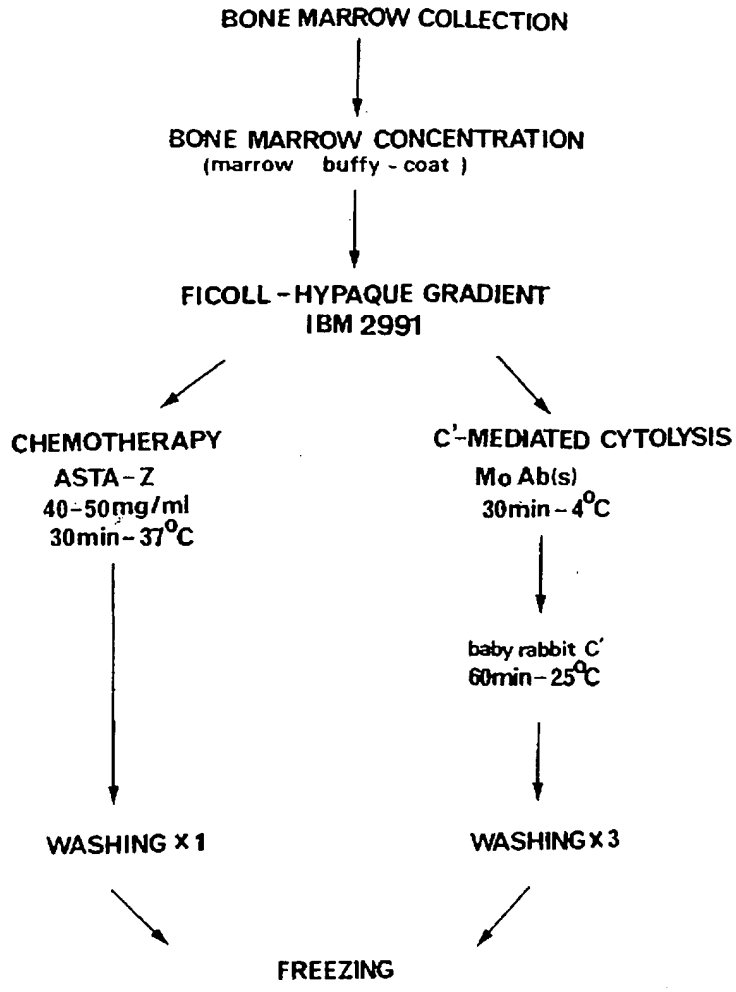

Fig 1. General protocol for *in vitro* treatment.

al, 1982; Herve *et al*, 1983). The marrow cells were layered on Ficoll-Metrizoate and the mononuclear cell layer washed three times using human serum albumin (4%).

For each treated marrow, an unpurged marrow rescue was put aside.

(b) *Ex-vivo treatment* (Fig 1). (i) *Exposure to a chemical agent* (Herve *et al*, 1984). We used Asta-Z-7557 mafosfamide (kindly provided by Asta-Werke Bidefeld, R.F.A.). For each patient (23 patients) we previously analysed marrow cell sensitivity to Asta-Z in a methyl-cellulose CFU-GM assay using human placenta as a source of colony-stimulating activity; in our Phase I assay, the dose of Asta-Z was increased from 10 to 50 μg/ml. The amount of Asta-Z-7557 calculated was added to the final suspension and adjusted to 1×10^7 cells per ml. The agitation time was 30 min in a 37°C waterbath with frequent gentle agitation. The cell suspension was then centrifuged at 6°C and the cell pellet was resuspended in plasma before freezing.

One T-ALL patient received marrow purged with deoxycoformycin (20 μM) (Prentice *et al*, 1980) associated with deoxyadenosine and inorganic phosphate (incubation time: 20 h at 37°C).

(ii) *Monoclonal antibodies and complement mediated cytolysis.* One or two anti-CALLA

MoAbs fixing rabbit C′ and included in the tenth cluster of differentiation, were used (CD 10: First (International Workshop on Leucocyte Differentiation Antigens): CD10/J5, Ig G_2 class from Coulter (Ritz *et al*, 1983), CD10/BA_3, Ig G_{2b} class from Hybritech (Kersey *et al*, 1984) and CD10/ALB_2 (Ig G_{2b} class) from Immunotech (Boucheix *et al*, 1984). Each time, the binding of MoAbs was assessed with fluoresceinated antimouse IgG (MELOY) using both fluorescence microscopy and flow cytometry (Ortho H50). Rabbit complement obtained from 23–28-d-old rabbits was used after having been extensively tested for toxicity against bone marrow progenitor cells. The suspension of mononuclear cells was incubated with one or two anti-CALLA MoAbs: J5 in three patients or AL B_2+BA_3 (two patients). We associated these two MoAbs because they do not recognize the same epitope on the antigenic molecule. MoAbs (2·5 μg of each one per 1×10^7 cells/ml) were simultaneously added to the mononuclear cell suspension adjusted at $2–3\times10^7$ cells/ml, incubated for 30 min at 4°C followed by complement mediated cytolysis (final dilution of 1:1) at 25°C for 60 min with a regular gentle agitation.

The rabbit C′ was removed by washing the suspension three times using a 4% human serum albumin. The cell suspension was then cryopreserved.

(c) *Freezing and thawing methods* have already been described (Herve *et al*, 1979). Briefly, dimethylsulphoxide (Me_2SO) was added to the cell suspension at a final concentration of 10%.

The marrow suspension was transferred into two Teflon Kapton freezing bags and the cooling rate was obtained with an electronic freezer (Nicool 4/G CFPO, Champigny s/Marne, France). The bags were stored in liquid nitrogen (−196°C). After thawing (42°C in a waterbath) the marrow cells were immediately reinfused through a peripheral vein 24–48 h after the end of the conditioning regimen.

(d) *Conditioning regimens.* For ANLL in CR1 the high-dose polychemotherapy consisted of 6-thioguanine, cytosine arabinoside, CCNU and cyclophosphamide (TACC modified) for 17 out of 18 patients (Herve *et al*, 1982). This protocol was previously used for ANLL in first relapse and we obtained six CR in six patients with a mean CR2 of 12 months for five patients (one patient died from herpes encephalitis during the fifth month of CR).

The treatment schedule was as follows: 6-thioguanine: 400 mg/m^2/d from day −6 to day −2; cytosine arabinoside 400 mg/m^2/d by continuous infusion from day −6 to day −2; CCNU 400 mg/m^2 on day −5 and cyclophosphamide 45 mg/kg/d from day −5 to day −2 for the first six patients and then lowered to 60 mg/kg/d (on days −3 and −2) because of the high cardiotoxicity of the regimen.

For a 3-year-old patient, the conditioning regimen was fractionated total body irradiation (TBI) followed by cyclophosphamide (60 mg/kg/d for 2 d).

All patients with ALL were conditioned with TBI followed by cyclophosphamide (60 mg/kg/d on days −3 and −2). In every case, lungs were protected over 10 Gy and cranial protection was considered according to the dose of previous irradiation. 14 out of 15 patients received fractionated TBI using the following protocol: 12 Gy in six fractions, during 3 consecutive days with a mean dose rate of 5–6 cGy/min using a linear accelerator.

Cyclophosphamide was administered at least 48 h after the end of TBI as in Sloan Kettering's Protocol (Shank *et al*, 1981).

In one case, a 7-year-old patient with an ALL_3, the conditioning high-dose chemotherapy was BACT (modified by the Institut Gustave Roussy) used in children B cell lymphomas (Hartman *et al*, 1982) association: 6-thioguanine (200 mg/m²/d from day − 5 to day − 2), cytosine arabinoside (200 mg/m²/d by continuous infusion from day − 5 to day − 2), cyclophosphamide (1·6 g/m²/d from day − 5 to day − 2) and BCNU (200 mg/m²/d on day − 6 to day − 4).

(e) *Supportive care.* 18 patients were isolated in a plastic isolator Isroom (La Calhène, France), 14 were treated in a protected environment. All patients received total gut decontamination with oral amikacin and vancomycin associated with miconazole or ketoconazole for fungal prophylaxy. Parenteral nutrition and transfusions were administered through a subclavian tunnelized silastic catheter (Silastic Vygon). The line was heparinized with low-dose heparin (1 mg/kg/d) to reduce the risk of catheter-related infection. All blood products were irradiated (45 Gy) prior to transfusion.

HLA compatible platelets were cryopreserved for immunized patients prior to transplant in the advent of the unavailability of fresh HLA compatible platelets.

RESULTS

(a) *Bone marrow storage*

The mean duration from CR1 to storage was 2 months for ANLL in first CR (range 0·6–6) and 3·3 months for ALL in first CR (range 2–4). For ALL autografted in CR2, three were stored in CR1 (mean 12 months from CR1) and eight in CR2 (mean 10 months from CR2).

The mean duration between storage and ABMT was 2·8 months (range 0·5–4) for ANLL and 4·3 months (range 0·5–29) for ALL patients.

(b) *Bone marrow collection characteristics*

An average $4{\cdot}23 \times 10^8 \pm 2{\cdot}38$ marrow nucleated cells per kg recipient weight was collected. The mean dose of CFU-GM collected was $15{\cdot}45 \times 10^4$/kg (range 2·7–42), $15{\cdot}2 \times 10^4$/kg for ANLL and $15{\cdot}7 \times 10^4$/kg for ALL.

Using the higher dose of Asta-Z (50 μg/ml) in 11 patients, the mean recovery of CFU-GM after *ex-vivo* treatment was 3·2%±2·6. The percentage of CALLA positive cells in the harvested marrow was 9·2% (range 1–26).

After incubation with anti-CALLA monoclonal antibodies and complement the final recovery of CFU-GM was 66·6%.

(c) *Haematopoietic recovery*

The mean recovery time of neutrophils over $0{\cdot}5 \times 10^9$/l was 27·4 d for ANLL (range 14–50) and 30 d for ALL patients (range 18–58).

The mean recovery time of platelets over 50×10^9/l was 46·3 d (range 21–100) for ALL

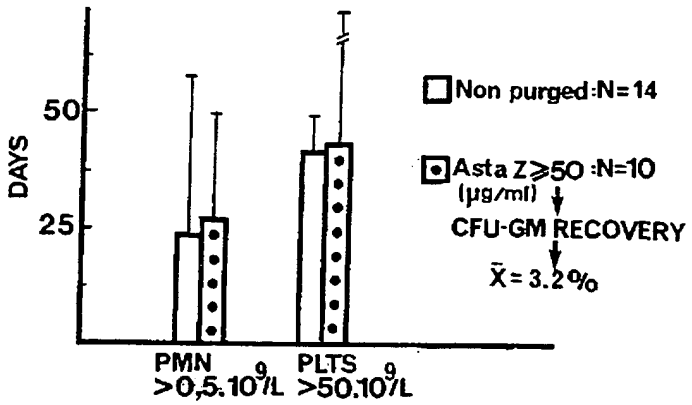

Fig 2. Effect of high-dose Asta-Z on haematopoietic recovery.

patients and one is still under 50×10^9/l with persistant platelet autoantibodies at day +190 post graft; for ANLL patients the mean time was 45·5 (range 13–90).

There was no significant difference in terms of haematopoietic recovery between non-purged and purged marrow with high-dose Asta-Z (Fig 2).

(d) *Transplant related complications*

Early complications. Six complications were lethal: three pericardiomyopathies following the TACC modified regimen (when we were using higher dosages of cyclophosphamide), one idiopathic interstitial pneumonitis (IP) and one aspergillus pneumonia in ANLL patients; one ALL patient with thrombocytopenia died of cerebral haemorrhage.

Ten out of 18 ANLL patients (55·5%) developed infections during the course of their aplasia (cf. Table I); only one was lethal (aspergillus pneumonia).

Seven out of 15 ALL patients developed serious infections (46·6%), none were lethal (cf. Table I). Most of the Gram-positive septicaemias were associated with mucositis.

Later complications. Five patients had later viral infections: four herpes zoster and one severe genital herpes; all of them were treated with acyclovir and none of them were lethal.

One 14-year-old patient with an ANLL in CR1 developed idiopathic interstitial pneumonitis 3 months after the graft.

Table I. Major infections during aplasia

ANLL	10/18 (55·5%)	6 septicaemia (5 Gram +; 1 Gram −) 1 aspergillus pneumonia 1 gastroenteritis (candida) 2 herpes mucositis
ALL	7/15 (46·6%)	5 septicaemia (3 Gram +; 2 Gram −) 1 bacterial pneumonia 1 bacterial cellulitis

Three later complications must be mentioned: one cataract, 2 years after TBI, and two premenopausal syndromes, one after TACC in a 40-year-old patient and one after TBI-Cy in a 27-year-old patient.

(e) *Follow up: ANLL patients* (cf. Table II)

At the time of analysis (October 1984), among 18 patients treated for ANLL in CR1, only 13 were evaluable because of five early deaths (27·8%): eight patients (44·4%) are alive and well in continuous complete remission (CCR) without any maintenance therapy: 47+, 47+, 30+ and five between 2·5+ and 8+ months post transplant (Karnofsky, 90–100%).

Five patients (27·7%) relapsed at 4, 7·5, 8, 11 and 18·5 months; three achieved a CR2 and remain in CCR with maintenance treatment, one is in partial remission with low-dose Ara-C, 2 months after relapse, and one still in relapse.

Table II. Current status of the acute leukaemia transplantation programme

	ANLL	ALL
No. of patients grafted	18	15
No. of evaluable takes	14	15
No. of autografted related deaths	5	1
No. of relapses	5	4
No. of CCR	8	10
No. of long CCR (>1 year)	3	5
No. of patients alive in relapse or subsequent CR	4	2

(f) *Follow up: ALL patients* (cf. Table II)

Among four patients treated for ALL in CR1, two adults are still in unmaintained long-term remission: one T-ALL patient with marrow purged with 2-deoxycoformycin (26+ months post graft) and one CALLA positive ALL patient transplanted with marrow purged with anti-CALLA MoAbs (12+ months post graft); one adult pre-T-ALL with marrow purged with Asta-Z who relapsed during the fourth month post graft, achieved CR2 with a rescue protocol and has remained in maintained CR2 for 11+ months; the child treated in CR1 for a Burkitt leukaemia relapsed during the eighth month post graft and died at 12 months.

Among 11 children treated for ALL in CR2, eight are alive and well in unmaintained CR with a mean follow up to 13·4 months post graft (range 2+–45+ months); two relapsed (9·8 and 5·3 months) and died; one patient died from cerebral haemorrhage (in CR) with prolonged thrombocytopenia.

DISCUSSION

One must first separate myeloblastic and lymphoblastic leukaemia as well as adult and childhood lymphoblastic leukaemia. A pilot study was started in 1980 for ANLL in CR1 conditioned with the TACC regimen; the toxicity of this combination was important at the beginning of the study because of the dosage of cyclophosphamide (as described by Gottdiener *et al.* 1981). After lowering the dose to 120 mg/kg no cardiac failure was observed in the next 12 patients conditioned with TACC. Eight patients out of 18 (44·4%) are still alive and well in CCR1. It should be pointed out that our three longest survivors were high risk ANLL at diagnosis (two with cytogenetic abnormalities and one with a high blast cell count). Two of them had been grafted with an unpurged marrow. According to the high toxicity of the TACC regimen, we have just started a similar study using factionated TBI followed by cyclophosphamide.

Published results in ANLL in CR1 (Table III) are heterogeneous: the timing of storage with respect to CR varies from author to author. Similarly, prognostic factors are not always specified. The First European Study from Gorin (1984) with 39 ANLL in CR1 from 15 centres appears to give more than 50% of unmaintained DFS, but the follow-up is still short; at the First International Symposium on ABMT held in Houston, Herve (1985) summarized the French survey with 37 patients treated for ANLL in CR (78% in CR1): 48·6% are DFS with 10 out of 18 for longer than 12 months. The Baltimore group (Santos & Kaizer, 1984), using 4-HC as a tumour purging agent, reports a study on nine patients with ANLL grafted in CR: two patients survived free of disease for 23+months (CR3) and 30+months (CR1).

In these various studies, and until now, there has not been a striking difference in terms of

Table III. ABMT in ANLL in CR1: summary of the literature

Centre	No. of patients	*In vitro* treatment	Conditioning regimen	Relapse	Follow-up (months)
Rotterdam (Löwenberg *et al.* 1984)	4	no	CY–TBI	1	3+, 18+, 45+
Glasgow (Burnett *et al.* 1984)	12	no	CY–TBI	5	21+, (6·5+–35+)
St Antoine (Laporte *et al.* 1984)	5	Asta Z	CY–TBI	1	3+, 9+, 10+, 11+
London (Anderson *et al.* 1984)	4	no	Dox, CY, BCNU 6-TG, Ara-C (double ABMT in CR1)	0	5+, 6+, 30+, 36+
Seattle (Stewart *et al.* 1983)	10	no	CY–TBI	4	5+, 7+, 27+, 32+

Abbreviations: CY: cyclophosphamide; TBI: total body irradiation; Dox: doxorubicin; 6-TG: 6-thioguanine; Ara-C: cytosine arabinoside.

haematopoietic recovery with or without marrow purging; moreover, the *in vitro* effect of 4-HC on the cellular immune response (Körbling *et al*, 1982) have shown a dose-dependent inhibition of lymphocyte proliferation to mitogen and alloantigen. In our study we did not find any difference in terms of immune recovery between marrow purged with Asta-Z and unpurged marrows (or purged with anti-CALLA). In the two groups we found an inverted ratio T4/T8 during the first 12 months and an altered cellular immunity lasting more than 1 year with functional tests (unpublished data).

For childhood ALL, high-dose cytoreductive therapy followed by ABMT has been proposed as an early intensification only in CR2, owing to very promising results of chemotherapy for childhood ALL in CR1 (Riehm *et al*, 1980). After relapse the prognosis remains poor and, in a recent study, the Children's Cancer Study Group (Baum *et al*, 1983) reports 30 patients with ALL in CR2 treated with chemotherapy, the median duration of remission is 14·2 months with 10 patients (33%) in CCR at 1 year and three (10%) at 2 years.

In our series, with a mean follow-up of more than 1 year, eight out of 11 children with ALL are still in unmaintained CR2 (Plouvier *et al*, 1985). Only two relapsed.

The Boston Group (Sallan *et al*, 1984) has published their clinical results on 19 patients autografted with purged marrows using CD10/J5 monoclonal antibody and complement: all patients were in second, third or subsequent remission: seven relapsed between 2 and 8 months, five died in CR from infectious or haemorrhagic complications, and seven are still in CR without maintenance therapy (four CR longer than 1 year).

The Minneapolis Group (Ramsay *et al*, 1984) has treated 21 patients with a combination of three MoAbs (CD10/BA3+CD9/BA2+CD24/BA1 and complement); 15 out of 21 relapsed, one died early and five are in CCR (range 1–37 months) with three longer than 1 year. The authors think it would be useful to intensify the conditioning regimen or suggest a maintenance therapy during a few months post ABMT.

In adult ALL we have autografted only three patients in CR1, two of them are still in long-term remission without any maintenance therapy.

Very few extensive studies have been published in adult ALL in CR1: in the Houston study (Vellekoop *et al*, 1984), 14 patients were conditioned with two cycles of CBV (CY, BCNU and VP-16). The median remission duration was 14 months: three patients relapsed after one course of treatment and five relapsed after the second course, four patients died after the second course, and only two patients remain alive and well in unmaintained remission with a total remission duration of 42+ and 47+ months. In The St Antoine Hospital in Paris (Laporte *et al*, 1984) four ALL have been consolidated in CR1 by TBI and cyclophosphamide followed by ABMT with marrow purged with Asta-Z; they are still in CR with a very short follow-up of 1+, 6+, 8+ and 10+ months. In the French survey (Herve, 1985) 27 patients conditioned by TBI+CY were autografted for ALL in CR (12 in CR1, 27 in CR2 and four in CR3): five relapsed (three among the 12 CR1) and 20 are currently in unmaintained CCR with a median duration of 7+months (range3+–21+months) from ABMT. Results are informative but the population is heterogeneous in terms of previous chemotherapy and the timing of transplant.

We think that, as in allogeneic transplants, the graft should be undertaken in the early

phase of ALL although recurrent leukaemia remains a problem (Thomas *et al*, 1983; Buckner & Clift, 1984; Jacobs & Gale, 1984; Zwaan *et al*, 1984).

The problem of purging methods has not yet been resolved: the efficacy of *ex-vivo* purging methods has been proven in some experimental models as in rat leukaemia (Sharkis *et al*, 1980) and in some experimental clonogenic studies using clonogenic cell lines such as HL-60 and malignant B cell lines (Stiff *et al*, 1984; Kalwinsky *et al*, 1983; Körbling & Hunstein, 1984). Results are somewhat controversial (Kluin-Nelemans *et al*, 1984) and their effectiveness has not yet been documented *in vivo*.

No specific monoclonal antibody is presently available for ANLL and the only well-developed purging technique remains exposure to chemical agents (4-HC, Mafosfamide). Controlled trials are therefore possible.

In a strategy for more effective ABMT in ALL, a complete elimination of human cells from marrow may be possible combining two purging methods. We are investigating the feasibility of *in vitro* treatment using a MoAb 'cocktail' specific for ALL associated antigens followed by exposure to a chemical agent such as Mafosfamide.

After Phase I–II studies of purging methods, prospective and comparative studies between unpurged and purged marrow are needed as well as a study of the ethical problems involved. In any case to avoid heterogeneous studies, we need larger series of homogeneous patients, conditioned with the same protocols and the same transplant timing. Moreover, in the near future, we shall have to compare the results of ABMT with the best chemotherapy available today and allogeneic BMT.

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Exhibit C

Terminal Differentiation Surface Antigens of Myelomonocytic Cells are Expressed in Human Promyelocytic Leukemia Cells (HL60) Treated With Chemical Inducers

By Bice Perussia, Deborah Lebman, Stephen H. Ip, Giovanni Rovera, and Giorgio Trinchieri

The expression of two surface antigens present on the cell membrane of both human granulocytes and monocytes was studied during the process of myelomonocytic differentiation using two monoclonal antibodies (B9.8.1 and B13.4.1). These surface antigens are not present on immature myeloid cells nor on nonmyeloid hematopoietic cells, but can be detected when the cells are terminally differentiated. Among the bone marrow cells, B13.4.1 binds to metamyelocytes and B9.8.1 to metamyelocytes and a fraction (30%) of myelocytes. HL60 human promyelocytic leukemia cells did not react with such monoclonal antibodies. However, when such cells were induced to differentiate in vitro into mature myeloid elements by treatment with retinoic acid or dimethyl sulfoxide, 70%–90% of the differentiated cells expressed both surface antigens. Cell sorting studies on these treated HL60 cells indicated that myelocytes and metamyelocytes were the most immature cells expressing such markers. Expression of the two surface antigens was also observed when HL60 cells were induced to differentiate into monocyte/macrophage cells by treatment with the tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate. Thus, human promyelocytic leukemia cells induced to differentiate in vitro by treatment with specific chemical agents express membrane antigens in the same pattern as normal bone marrow myeloid cells at the corresponding stage of differentiation.

LITTLE IS KNOWN about the changes that occur on the membrane surface during the differentiation of myelomonocytic precursor cells to granulocytes and monocytes/macrophages, and few reagents are presently available that recognize specific surface antigens of myelomonocytic cells. Studies on complement receptors (CR) have shown that CR1 (the immune-adherence C4b-C3b receptors that are present on membranes of both granulocytes and monocytes) appear relatively late during myeloid differentiation and can be detected in a fraction of metamyelocytes and in almost all band cells and granulocytes.[1] The receptors for the Fc fragment (FcR) of IgG molecules (IgG-FcR) appear earlier than CR1 during terminal differentiation of myeloid cells. While absent on myeloblasts, FcR are detected on about half of the promyelocytes and almost all the myelocytes.[2] Despite the fact that monocytes and granulocytes have a common stem cell,[3] the intermediate progenitor cells of the monocyte/macrophage lineages are not clearly identifiable in the bone marrow, and the kinetics of CR1 and IgG-FcR appearance on differentiating cells of the monocytic lineage is not known. The only information available in this area concerns the disappearance of surface DR antigens at the promyelocytic stage, and the expression of the same antigens at the surface of about 80% of the monocytes but not of the granulocytes.[4]

CR1, IgG-FcR, and DR antigens, however, are not markers specific for any one cell type; CR are also found on erythroid cells and B lymphocytes; IgG-FcR are present on platelets, some subsets of lymphocytes, and eosinophils; and DR antigens are found on B and pre-B lymphocytes and have been described on several other cell types.[4]

The use of monoclonal antibodies produced by somatic cell hybridization technique[5] has allowed the identification of defined membrane molecules[6] in studies of lineage-specific surface markers. A monoclonal antibody that reacts with mouse macrophages and, to a lesser extent, with monocytes and granulocytes has been described,[7] as well as antibodies that recognizes surface antigens present on human monocytes and granulocytes.[8,9] At the present time there are no studies directed to identify whether such surface antigens are expressed only on terminally differentiated cells or are found on all mature and immature cells of myelomonocytic lineage.

Recently we have obtained several monoclonal antibodies directed to human cells of the myelomonocytic lineage. While many of these monoclonal antibodies recognize surface antigens present throughout all stages of the myelomonocytic differentiation, two of these antibodies (B9.8.1 and B13.4.1) recognize surface antigens present only on terminally differentiated cells.

The expression of these antigens has been investigated in an in vitro model of myelomonocytic differentiation. When human promyelocytic leukemia cells (HL60)[10] were induced to differentiate in vitro into mature metamyelocytes and granulocytes by treat-

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ment with dimethyl sulfoxide[11] and retinoic acid,[12] or into macrophage-like cells by treatment with 12-0-tetradecanoyl-phorbol-13-acetate (TPA),[13,14] they expressed these two late differentiation markers. The induction of differentiation of human myeloid cell lines in vitro leads to the expression of surface antigens detected by the two monoclonal antibodies and present in vivo on normal and myeloid leukemia cells at the corresponding stage of differentiation.

MATERIALS AND METHODS

Cells and Cell Culture

All the cell lines used in the experiments reported here were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), glutamine, 100 U penicillin/ml, and 100 μg streptomycin/ml. The mouse myeloma cell line P3-X63-Ag8 and the hybrid cell lines were grown in Dulbecco's modified minimal essential medium containing 10% heat-inactivated gamma-globulin-free horse serum.

Normal human mononuclear cells were obtained from peripheral blood of healthy donors after removal of granulocytes and erythrocytes by centrifugation on Ficoll/Methrizoate gradient (Lymphoprep, Nyeggard, Oslo, Norway).[15] The mononuclear cell fraction was allowed to adhere on plastic (1 hr at 37°C). Monocytes were recovered by scraping the flasks, and pure lymphocytes were obtained from the nonadherent fraction after removal of the few contaminating phagocytic cells by the carbonyl iron method.[16] Polymorphonuclear cells were obtained by sedimentation of total blood with Dextran and subsequent centrifugation of the buffy coat on Ficoll/Methrizoate gradient. The purity of the different cell fractions was always over 98% as judged by Wright and α-naphthyl acetate esterase staining (αNAE).[17] Normal bone marrow cells were prepared from diagnostic bone marrow aspirates obtained from nonleukemic patients. The cells were purified on a Ficoll/Methrisoate gradient (density 1.078 g/ml). Erythrocytes were thus eliminated, and only a small percentage of the mature granulocytes still contaminated the cell population at the interface.

Monoclonal Antibodies

BALB/c mice were immunized (one single injection i.v.) with 3×10^7 human normal peripheral blood leukocytes obtained from buffy coats from defibrinated venous blood of healthy donors. Three days after the injection, 10^8 spleen cells were fused with 10^7 P3-X63-Ag8 cells. Fusion, selection of positive hybrids, cloning, and production of ascites were carried out as previously described.[18] The monoclonal antibodies described here were obtained from two independent fusions.

Both the monoclonal antibodies described in this paper were IgM as determined by immunodiffusion and indirect binding assay with class-specific antibodies as previously described.[18] B9.8 clone 1 hybrid cells secreted an excess of the parental myeloma γ_1 chain, the μ chain, and two light chains. B13.4 clone 1 secreted only μ heavy chain and two light chains. Purified IgM antibodies were obtained from the ascites fluid by gel-filtration on Sephadex G-200 (Pharmacia Fine Chemicals, Uppsala, Sweden).

Detection of Antigens Recognized by Monoclonal Antibodies

^{125}I-labeled rabbit F(ab')$_2$ anti-mouse F(ab')$_2$ was used in the indirect radioimmunoassay, performed as previously described.[17]

Competition experiments were performed in order to define whether the two monoclonal antibodies were reacting with the same or discrete antigenic structures. Target cells (10^6) were incubated with 20 μl of cold supernatant fluid that had been concentrated 20-fold and 20 μl of purified antibody labeled with ^{125}I-Bolton-Hunter reagent (Amersham, England). After 30 min incubation at 4°C the cells were washed four times, and the bound radioactivity in the pellet was counted in a gamma counter. Control P3-X63-Ag8 supernatant and autologous supernatant were included in all the tests.

Indirect immunofluorescence was performed according to Loor et al.[18] A fluorescein-conjugated goat F(ab')$_2$ anti-mouse F(ab')$_2$ immunoglobulin (Cappel, Cochranville, Pa.) was used throughout.

For immunoperoxidase staining, cytocentrifuge preparations of the cells were fixed in formaldehyde acetone (pH 6.6), and endogenous peroxidase was blocked by reaction with 7.5% H_2O_2 for 10–30 min at room temperature. The slides were washed in phosphate-buffered saline, 0.1% gelatin–0.1% NaN_3, and incubated with the antibody for 15 min at room temperature. After washing, the slides were sequentially incubated with a goat anti-mouse Ig antiserum (Cappel) and a peroxidase-conjugated rabbit anti-goat IgG antiserum (Cappel). All incubations were performed at room temperature for 15 min. After reaction, the slides were incubated for 10 min with substrate solution [20 mg amino-3-ethyl-9-carbazol (Sigma, St. Louis, Mo.) were dissolved in 2.5 ml N,N-methyl-formamide (Sigma), and added to 50 ml of 50 m*M* sodium acetate buffer (pH 5.0)]. Slides were counterstained with hematoxylin to detect the morphology of the positive cells.

The technique of Goding[19] was used for rosette formation. Cells in suspension were treated with the monoclonal antibody and then with a rabbit F(ab')$_2$ anti-mouse F(ab')$_2$ fragment (Cappel). Antibody-treated cells were incubated 30 min at 4°C with sheep red blood cells (SRBC) coated with goat IgG anti-rabbit IgG (H + L chains, Cappel). Cells that bound 5 or more SRBC were considered positive; their morphology was detected by Wright staining.

Table 1. Reactivity of the Monoclonal Antibodies B9.8.1 and B13.4.1 on Different Human Cell Types as Detected by Immunofluorescence

Cell Type	B9.8.1	B13.4.1
Granulocytes†	>95*	>95
Mononuclear blood cells†	14	10
Lymphocytes†	0	0
Monocytes†	>95	>95
Platelets†	0	0
Erythrocytes†	0	0
Thymocytes‡	0	0
K562 erythromyeloid leukemia line	0	0
KG1 myeloid leukemia line§	0	0
HL60 myeloid leukemia line	<10	<5
B-lymphoid lines (8 tested)	0	0
T-lymphoid lines (5 tested)	0	0
Fetal fibroblast strains (4 tested)	0	0
Tumor-derived adherent cell lines (11 tested)‖	0	0

*Percent positive cells by indirect immunofluorescence.

†Obtained from peripheral blood of several normal blood donors, as described in Materials and Methods.

‡Obtained from fragments of thymus removed from 4 children undergoing heart surgery.

§Obtained from Dr. H. P. Koeffler (University of California at Los Angeles).

‖Six melanoma, 3 colon-carcinoma, 1 cervix carcinoma, and 1 rhabdomyosarcoma derived cell lines.

Induction of Differentiated HL60 Cells

TPA (Peter Borchert, Eden Prairie, Minn.) was dissolved in acetone (1 mg/ml), and RA (Fisher) in ethanol (0.3 mg/ml). HL60 cells (2×10^5/ml) were cultured with 10^{-7} *M* RA, 1.2% DMSO, or 1.6×10^{-8} *M* TPA as previously described[10-12] and collected at different days after induction. Morphology of the induced cells was determined by Wright-Giemsa staining. In our hands, maximal differentiation of HL60 after exposure to DMSO occurred on day 5, as demonstrated both by morphological and functional criteria.[13,21] The Cytofluorograf System 50H connected to a Data General MP/200 microprocessor (Ortho Instruments, Westwood, Mass.) was used for fluorescence analysis and cell sorting experiments.

RESULTS

Identification of the Cells in Peripheral Blood and in Normal Bone Marrow That React With Two Monoclonal Antibodies

Using several different techniques, only monocytes and granulocytes (among the peripheral blood cells)

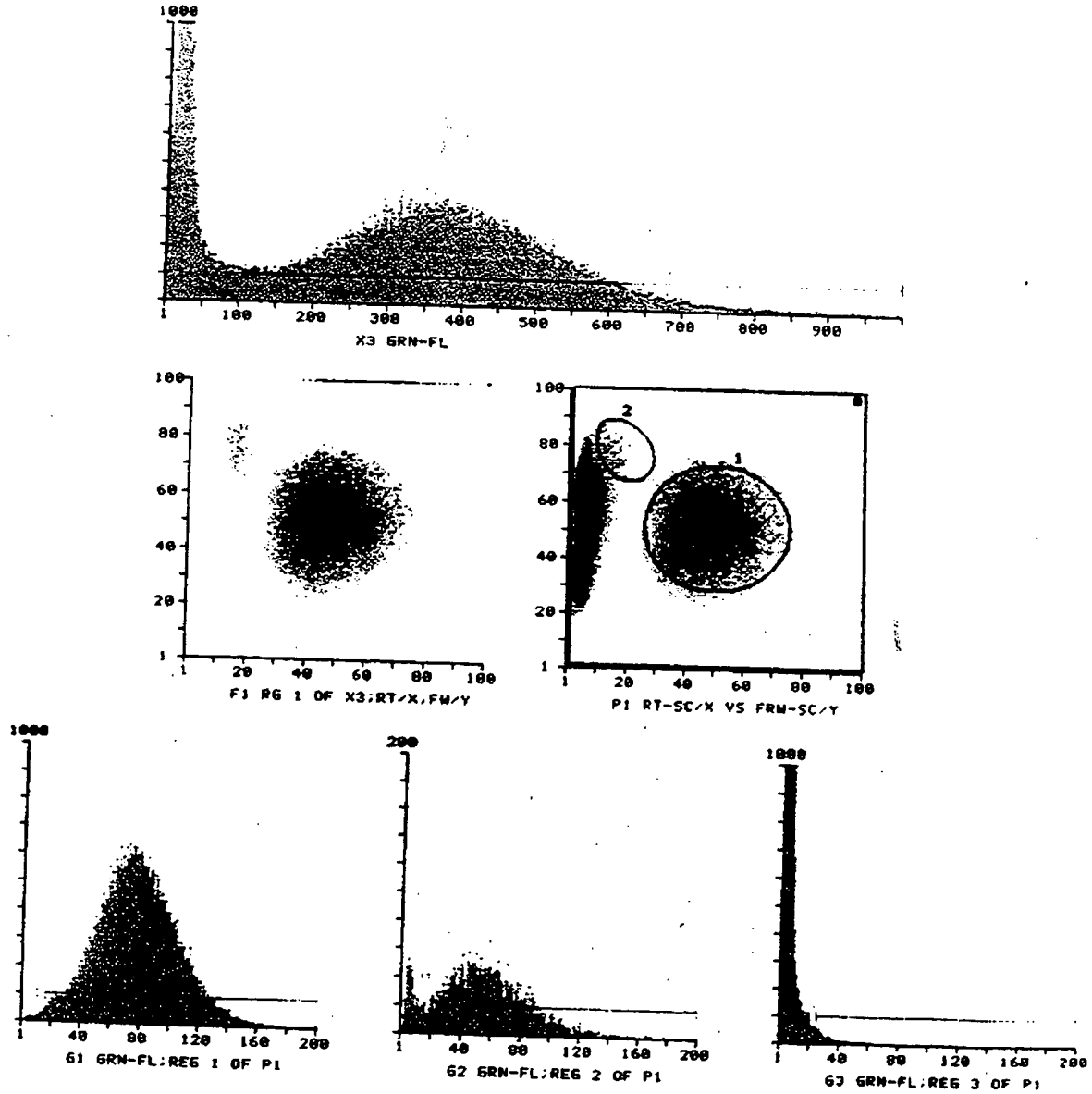

Fig. 1. Reactivity of B13.4.1 monoclonal antibody with human leukocytes. Human buffy coat cells from peripheral blood were incubated with B13.4.1, washed, stained with FITC-labeled goat F(ab')$_2$ anti-mouse Ig and analyzed in an Ortho Cytofluorograph 50H. X3 shows the fluorescence of all cells. The vertical bars (1 —) indicated the threshold of fluorescence at which 99% of the cells treated with control supernatant from the parental myeloma and FITC anti-mouse Ig were negative. P1 shows the light scattering analysis of all cells, and F1 shows the light scattering analysis of the cells positive for immunofluorescence and included in region 1 of histogram F1. Regions 1, 2, and 3 of P1 correspond to granulocytes, monocytes, and lymphocytes, respectively.[20] In F1 only the positive granulocytes and monocytes, but not the negative lymphocytes, are displayed. Histograms G1, G2, and G3 show the analysis of fluorescence in region 1 (granulocytes), 2 (monocytes), and 3 (lymphocytes) of scattergram P1. Parameters: Histograms X3, G1, G2, G3—ordinate number of cells, abscissas arbitrary units of fluorescence. Scattergrams P1 and F1—ordinate forward angle light scatter, abscissas right-angle light scatter.

were recognized by the B9.8.1 and B13.4.1. Of a large panel of B and T lymphoid cell lines, normal and tumor-derived adherent cell lines and myeloid cell lines, only 0%–10% positive cells were detected on HL60 by immunofluorescence (Table 1). These results have been confirmed by radioimmunoassay, immunofluorescence, and by immunoperoxidase techniques. Using the last two techniques, the majority of granulocytes and monocytes were found to react with both antibodies. Eosinophilic granulocytes were found to be negative for both antigens. Radioimmunoassay and immunofluorescence studies indicate that B9.8.1 reacts more strongly on granulocytes than on monocytes, whereas B13.4.1 reacts to a similar extent with both cell populations. To test the possibility that the two monoclonal antibodies react with the same antigen, experiments to measure competitive inhibition of binding were performed using a large excess of cold antibody. The binding of each of the two antibodies was completely inhibited by the presence of the cold homologous antibody, but no cross-inhibition was observed (data not shown), indicating that the two antibodies do not react with the same antigenic determinants. Analysis of the blood cells using the cell sorter confirmed the data obtained with the other procedures. Data obtained using B13.4.1 monoclonal antibody are shown in Fig. 1.

The stages during myeloid differentiation at which the two antigens appear were studied using the immunoperoxidase technique on specimens from normal bone marrow aspirates. The results are shown in Fig. 2A. B9.8.1 reacted with a fraction of myelocytes and with more mature cells, whereas the antigen recognized by B13.4.1 antibody was expressed on cells at a later stage of differentiation, and only metamyelocytes, band cells, and granulocytes reacted with the antibody. Bone marrow samples were also analyzed by the indirect rosetting technique, and comparable results on the distribution of the surface antigens recognized by the two monoclonal antibodies were obtained. Erythroid cells and megakaryocytes did not react in any case. Positive and negative cells have also been separated using a fluorescence activated cell sorter: the results of sorting experiments confirmed the specificity pattern observed by immunoperoxidase and indirect rosetting (Table 2). A few cases of leukemias were also tested (Table 3). Cells positive for B9.8.1 and B13.4.1 were found only in cases where mature myelomonocytic cells were present, i.e., in acute myelomonocytic and chronic myeloid leukemia. Leukemic cells from patients with acute lymphoid or myeloblastic leukemia were negative. The distribution of the antigens on the different cell types was also analyzed by immunoperoxidase staining in one case of chronic myeloid leukemia (Fig. 2B). The results correspond to those obtained with normal bone marrow cells.

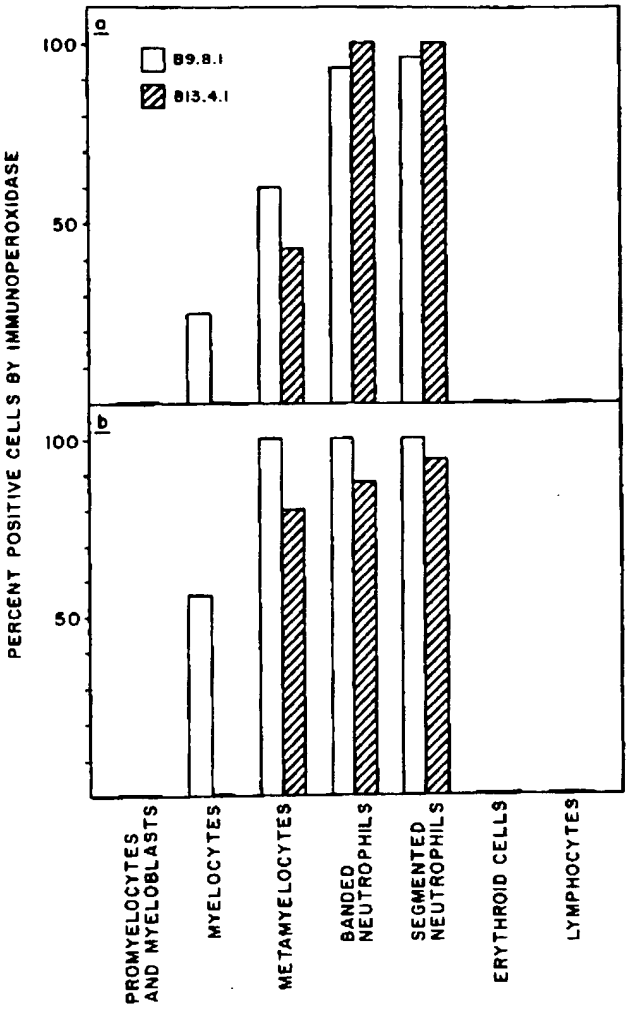

Fig. 2. Proportion of bone marrow cells bearing the antigens recognized by the monoclonal antibodies B9.8.1 and B13.4.1 as detected by immunoperoxidase technique. Morphological identification of the cells was based on counterstaining with hematoxylin. (A) Bone marrow cells from a normal subject. (B) Bone marrow cells from a patient affected by chronic myeloid leukemia.

Expression of the Antigens Recognized by B9.8.1 and B13.4.1 on HL60 Cells Induced to Differentiate In Vitro

After defining the stage at which the two surface antigens are expressed in human bone marrow cells, we investigated whether the expression of the antigens could be induced during the process of myelomonocytic differentiation in vitro. The promyelocytic cell line HL60 was induced to differentiate in vitro either to macrophage-like cells by treatment with TPA or to metamyelocytes and granulocytes by DMSO or RA treatment. After induction, the cells were tested daily

Table 2. Sorting of Positive and Negative Cells for B13.4.1 From Bone Marrow Cells Using a Fluorescent-Activated Cell Sorter

Cell Type	Negative (%)	Positive (%)
Myeloblasts	1	0
Promyelocytes	12	2
Myelocytes	23	2
Metamyelocytes	7	41
Granulocytes	0	35
Proerythroblasts	3	0
Basophilic erythrocytes	3	0
Polychrom erythrocytes	17	0
Orthochromatic erythrocytes	28	3
Monocytes	0	14
Lymphocytes	17	3

Normal bone marrow cells, separated on a discontinuous Ficoll-Hypaque gradient (density, 1.078 g/ml), were stained with B13.4.1 by indirect immunofluorescence.

Positive and negative cells were sorted in a Cytofluorograph 50H cell sorter.

Five-hundred positive and 500 negative cells have been morphologically identified after Wright-Giemsa staining. Numbers represent the percent of the cell types within each sorted population.

Table 4. Kinetics of Appearance of the Antigens on Chemically Induced HL60 Cells

Experiment	Cells	Inducer	Day of Treatment	B9.8.1	B13.4.1
1	HL60	None	0	0*	0
		TPA	1	440	897
			2	1,236	2,859
			3	909	2,270
			4	1,359	4,234
	Monocytes	None	0	971	2,450
	Granulocytes	None	0	3,273	3,229
2	HL60	None	0	127	27
		DMSO	1	0	0
			2	727	271
			3	656	603
			4	646	281
			5	1,055	823
	Monocytes	None	0	628	3,591
	Granulocytes	None	0	3,168	4,789
3	HL60	None	0	214	0
		RA	5	2,399	726
	Granulocytes	None	0	2,641	5,157

*Numbers represent cpm obtained in indirect radioimmunobinding with the antibody minus cpm obtained by incubation of the cells with supernatant from parental myeloma.

for the expression of B9.8.1 and B13.4.1 antigens by indirect radioimmunobinding. Following treatment with TPA, both antigens appeared. Both antigens could be detected as early as day 2 after induction and were fully expressed on the cells by day 4, when almost all of the cells had the morphological, enzymatic, and surface markers (CR1, Fc receptors) characteristic of differentiated macrophages.[20] The antigen recognized by B13.4.1 was expressed on the TPA-induced HL60 at a level similar to or even higher than that on peripheral blood monocytes and granulocytes (Table 4). In contrast, the binding of B9.8.1 to TPA-induced cells is comparable to that observed on peripheral blood monocytes but much lower than that on peripheral blood granulocytes.

Both DMSO and RA treatment also induced both surface antigens, with a maximum on day 5 and with a similar pattern of expression. The expression of the antigen recognized by B13.4.1 on DMSO- and RA-treated cells, at difference with that observed with TPA induction, was induced at a much lower level than that observed on differentiated peripheral blood myelomonocytic cells. B9.8.1 reacted with RA-induced cells and with peripheral granulocytes to a similar extent (Table 4). The presence of the antigens on DMSO-treated cells was not followed after 5 days, since in our experimental conditions it represented maximal differentiation.

Similar results were obtained in cell sorting experiments. Untreated HL60 cells were allowed to react with each of the antibodies and run through the Ortho Cytofluorograf 50H. Less than 10% of the untreated HL60 cells and 80% of the TPA-treated cells were positive with both antibodies; RA-treated cells were

Table 3. Proportion of Cells Positive With the Monoclonal Antibodies B9.8.1 and B13.4.1 in the Bone Marrow From Normal and Leukemic Subjects

	Number of Cases	B9.8.1	B13.4.1	Monoclonal Anti-DR*	E-RFC	EA7S-RFC
Normal†	3	16–26†	18–30†	29–45†	22–28§	14–36‖
B-acute lymphatic leukemia	9	0–5	0–10	80–100	0–9	0
T-acute lymphatic leukemia	4	0	0	0–5	60–90	0
Acute myeloid leukemia	3	0–10	0–10	10–80	0	0–5
Acute myelomonocytic leukemia	1	0	70	100	0	50
Chronic myeloid leukemia	1	65	59	3	0	35

*Monoclonal antibody B33.1.1 reacting with a nonpolymorphic determinant of the human HLA-DR antigens (Perussia and Trinchieri, unpublished).

†Bone marrow cells were in all cases purified on a Ficoll-Hypaque gradient (density, 1.078 g/ml).

†Percent cells positive by indirect immunofluorescence.

§Number of cells forming rosettes with SRBC.

‖Number of cells forming rosettes with ox erythrocytes coated with rabbit IgG anti-ox antibodies.

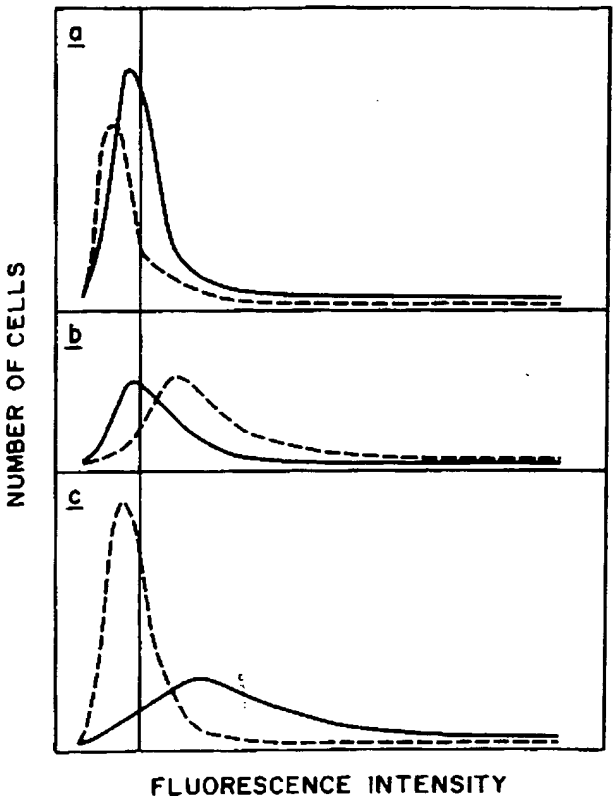

Fig. 3. Induction of myeloid surface antigens on HL60 cells treated with chemical inducers, as analyzed by flow cytofluorimetry. (A) Control noninduced HL60 cells. (B) Cells treated with 10^{-7} *M* RA for 5 days. (C) Cells treated with 1.6×10^{-8} *M* TPA for 3 days. (----) B9.8.1 monoclonal antibody, (—) B13.4.1 monoclonal antibody. The vertical continuous line indicates the threshold fluorescence intensity at which 95% of the cells treated with control supernatant from the parental myeloma were negative.

65% positive with B13.4.1 and 90% positive with B9.8.1. Fluorescence was more intense when RA-treated cells were stained with B9.8.1 than with B13.4.1, and the reverse was true for the TPA-treated cells (Fig. 3). In one experiment, the cells positive for either B9.8.1 or B13.4.1 antibody on day 3 after induction with RA were sorted and examined for their morphology. In agreement with our results using bone marrow cells, only cells with the morphology of metamyelocytes and granulocytes were obtained in the fractions that were fluorescent with either antibodies.

DISCUSSION

We have defined the stages during human myelomonocytic differentiation at which two surface antigens, recognized by the monoclonal antibodies B9.8.1 and B13.4.1, are expressed. These monoclonal antibodies are not only lineage-specific, but they recognize myelomonocytic cells at a precise stage of differentiation. Mature myelomonocytic cells but not hematopoietic cells of other lineages or nonhematopoietic cells express these antigens.

So far, characterization of cells of the myelomonocytic lineage in humans has been based on morphological, cytochemical, and functional properties, and on the presence or absence of surface receptors. The use of specific monoclonal antibodies in characterizing cells at different stages of differentiation adds a new dimension and provides an increased specificity over other techniques currently available.

The distribution of the cells positive for the monoclonal antibodies B9.8.1 and B13.4.1 was analyzed using normal bone marrow cells or peripheral blood of patients with chronic myeloid leukemia. The proportion of positive cells in both cases is close to the proportion of the more mature myeloid cells present in the samples. Cells positive with B9.8.1 were identified by immunoperoxidase staining and indirect rosetting as myelocytes or more mature cells, whereas the cells positive for B13.4.1 were metamyelocytes, band cells, and granulocytes. The small percentage (0%–10%) of HL60 cells that are positive by immunofluorescence with both the antibodies probably reflects the presence of a small proportion of metamyelocytes derived from spontaneously differentiating cells within the HL60 cell population.[11]

Since both B9.8.1 and B13.4.1 are IgM immunoglobulins, it is unlikely that the results on the specificity of the antibodies are altered by aspecific absorption of the antibodies on the cells via their Fc fragment. Moreover, $F(ab')_2$ fragments of the second antibody were used, and control samples incubated with the supernatant from the parental myeloma line, which contains IgG1, were negative. It is not clear yet whether the two monoclonal antibodies recognize two different molecules on the surface membranes of the cells or two different antigenic sites on the same molecule that become exposed at different times during differentiation. By competition experiments on positive cells, no crossinhibition was observed between the two monoclonal antibodies. Immunoprecipitation studies of the molecules reacting with such antibodies have so far been inconclusive.

The absence of reaction of B9.8.1 and B13.4.1 with all the B-cell lines tested, with peripheral blood lymphocytes, and with human erythrocytes indicated that the antibodies are not directed against complement receptors. The binding of complement-sensitized erythrocytes by CR-bearing myeloid cells, moreover, is not inhibited by B9.8.1 and/or B13.4.1 (data not shown). The possibility of reaction with receptors for immunoglobulins cannot be formally ruled out. FcR, as well as the antigens recognized by B9.8.1 and B13.4.1, were present on HL60 cells after induction of

differentiation in vitro. Furthermore, the lack of reactivity with lymphocytes and K562 cells cannot be considered as evidence against a reactivity with FcR, as the FcR present on myelomonocytic cells may differ from those present on these two cell types.[18] However, lymphokines can induce the expression of FcR on HL60 cells in the absence of both morphological differentiation and induction of B9.8.1 and B13.4.1 antigens (Perussia and Trinchieri, unpublished). No correlation was shown between the reactivity with either antibody and the presence of DR antigens on both immature and terminally differentiated cells of the lymphoid and myelomonocytic lineages. Moreover, DR antigens were not induced on HL60 cells upon TPA treatment (data not shown) as opposed to normal macrophages. The two monoclonal antibodies, therefore, recognize surface structures not previously defined.

The kinetics of appearance of the markers recognized by B13.4.1 and B9.8.1 were investigated using the human promyelocytic leukemia cell line HL60 induced to differentiate in vitro along the myelomonocytic lineage. Induction by DMSO, RA, or TPA causes the expression of CR1 and IgG-Fc receptors on these cells.[21] Other markers of terminal differentiation of myeloid and of monocytic lineage are expressed on the HL60 cells induced to differentiate by the chemical inducers; DMSO-induced HL60, however, do not develop alkaline phosphatase or lactoferrin, two typical markers of mature granulocytes.[24,25] The expression of the antigens recognized by the two antibodies on RA-induced HL60 cells was compatible with the observations made on the normal marrow: cell sorting experiments indicated that the RA-induced HL60 cells that are positive for the B9.8.1 antibody morphologically resemble myelocytes or more mature cells, whereas B13.4.1 recognizes an antigenic determinant present only on metamyelocytes and segmented cells.

B13.4.1 reacted more strongly with HL60 cells induced by TPA to differentiate into monocytes/macrophages than on DMSO- or RA-induced cells, and its expression was quantitatively similar to that of peripheral blood monocytes. On DMSO- and RA-induced HL60 cells, the antigen recognized by the B9.8.1 antibody was expressed at a level quantitatively similar to those of peripheral blood granulocytes, whereas the antigen recognized by B13.4.1 was expressed at a much lower level. These findings indicate that the process of induction of differentiation of HL60 cells either by DMSO or RA in vitro appears to be qualitatively similar to the process of myeloid differentiation in vivo, but quantitative expression of the surface markers may be different. When, during TPA induction, the kinetics of appearance in vitro of the surface antigens recognized by the two monoclonal antibodies is compared to that of other marker events (surface structures, enzymes, and functions), a well defined time-course of events spanning 72 hr can be established, possibly reflecting the in vivo sequence of events in the monocyte-macrophage differentiation process.[21] No direct correlation between the appearance of the two surface markers and the development of differentiated myeloid function can yet be stated. However HL60 cells and other myeloid cell lines recently established will represent a useful in vitro model system to analyze in more detail the molecular events occurring at the surface membranes of cells of the myelomonocytic lineage.

ACKNOWLEDGMENT

We thank Dr. Stephan Carrel (Ludwig Institute for Cancer Research, Epalinges, Switzerland) for providing some of the lymphoid and nonlymphoid cell lines used to test the monoclonal antibodies; Dr. B. Lange (The Children's Hospital of Philadelphia, Philadelphia, Pa.) for providing bone marrow and peripheral blood specimens from leukemic patients; Marina Hoffman for editing the manuscript; and Ann McNab for secretarial assistance. The two monoclonal antibodies described here were produced and partially characterized when B.P. and G.T. were at the Unit of Human Cancer Immunology, Ludwig Institute for Cancer Research and at the Unit of Genetics, Swiss Institute for Experimental Cancer Research, Switzerland, respectively.

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Exhibit D

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EP Patent No. 0 403 506
Application No. 89 90 2686.8
"Method of screening for protein inhibitors ..."
Our Ref.: A 205 EP

April 30, 1999
Jae/PST/fp

Dear Gerard,

Enclosed please find the Opposition Division's summons to attend oral proceedings on

December 9, 1999 at 9 a.m.

The term for making written submissions and/or amendments expires on October 8, 1999.

Furthermore, the provisional opinion of the Opposition Division is set out in the Communication accompanying the summons. In this respect, the Opposition Division is of the opinion that claim 1 is not clear with respect to feature (c) and suggests replacing the term "a responsive change in said phenotypic characteristic" with the term "a responsive change in said phenotypic response"; see section 12.a of the Communication.

Please let us know whether you can agree to the assessment of the Opposition Division and, if so, whether in your view a corresponding change in the terms of feature (c) is derivable from the teaching of the application as filed. At first glance, it appears to us as if the Opposition Division's notion is not entirely unjustified. For example, according to the wording of the dependent claims or claim 3, the phenotypic response rather than the phenotypic characteristic is evaluated in order to determine whether a substance is an inhibitor or activator.

We would appreciated receiving your comments in this respect.

Apart from its objection under Article 84 EPC to claim 1, the Opposition Division is of the provisional opinion that the grounds opposition would not prejudice maintenance of the patent provided that the objections under Article 84 mentioned above are dealt with satisfactorily.

We would appreciate receiving your comments on the above in due course.

If you have any questions on the Communication, please do not hesitate to contact us.

With best regards,

Dr. Hans-Rainer Jaenichen

cc: Dr. Lee, Kenyon and Kenyon; Dr. Stadheim, Stadheim & Grear

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A 205 EP	89902686.8-2116/0403506

Anmelder/Applicant/Demandeur/Patentinhaber/Proprietor/Titulaire
HOUSEY, Gerard M.

SUMMONS TO ATTEND ORAL PROCEEDINGS PURSUANT TO RULE 71(1) EPC

You are hereby summoned to attend oral proceedings arranged in connection with the above-mentioned European patent.
The matters to be discussed are set out in the communication accompanying this summons (EPO Form 2906).

The oral proceedings, which will be public, will take place before the opposition division

****
* on 09.12.99 at 09h 00 hrs in Room 3466 at the EPO Bayerstr.34 *
* PschorrHöfe, D-80335 München *
****

The time and date have already been agreed with you. No further changes in the arrangements can now be made, except on serious grounds.
If you do not appear as summoned, the oral proceedings may continue without you (Rule 71(2) EPC).
Your attention is drawn to Rule 2 EPC, regarding the language of the oral proceedings, and to the Official Journal 9/91, p. 489, concerning the filing of authorisations for company employees and lawyers acting as representatives before the EPO.
The final date for making written submissions and/or amendments (Rule 71a EPC) is 8.10.99.

You are requested to report in good time beforehand to the porter in the EPO foyer. Room 3473 and 3474 are available as waiting rooms.
Parking is available free of charge in the underground car park.
However, this applies only in the case of accessing the car park via the entrance "Zollstrasse".

For the opposition division:
Tel. No.: (089) 2399- 8164
A.W. Ormerod

Annexes:
Confirmation of receipt (Form 2936)
~~Rule 2 EPC~~ (~~EPO Form 2043~~)
Communication (EPO Form 2906)

Registered letter with advice of delivery

1). This communication contains provisional and not binding opinions based on the documents on file.

2). Three oppositions have been filed in respect of patent **EP-B-0 403 506** granted to **HOUSEY, Gerard M.** (hereafter referred to as the "*Proprietor*"). Said patent is based upon European patent application No. 89902686.8, filed on 09.02.1989, claiming a priority date of 10.02.1988. The title of the patent is "*Method of screening for protein inhibitors and activators*". Said application is derived from an international application filed under the PCT and published under the international publication number WO 89/07654.

3). The Opponents are:

Opponent 1 (O1): **Hoechst AG**

Opponent 2 (O2): **Boehringer Mannheim GmbH**

Opponent 3 (O3): **Boehringer Ingelheim GmbH**

4). In preparing the present communication the Opposition Division has taken into consideration the following documents:

o **sent by Opponent 1**:

- notice of opposition dated 26.03.1997 as re-filed with the letter of 01.04.1997 and received on 03.04.1997

o **sent by Opponent 2**:

- notice of opposition dated 09.04.1997 as received on 10.04.1997

o **sent by Opponent 3**:

- notice of opposition dated 07.04.1997 as received on 09.04.1997

- **sent by the Proprietor**:

  - the observations dated 08.04.1998 as received on the same date

5). The oppositions have been based on the grounds that:

- As regards each of Opponent 1 and Opponent 2:

  - the subject-matter of the patent is not new and does not involve an inventive step (**Article 100(a) EPC**), and

  - the subject-matter of the patent extends beyond the content of the application as originally filed (**Article 100(c) EPC**).

- As regards Opponent 3:

  - the subject-matter of the patent is not new and does not involve an inventive step (**Article 100(a) EPC**), and

  - the patent does not disclose the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art (**Article 100(b) EPC**)

6). The present requests of the parties are the following:

a) As regards the Opponents

Revocation of the parties in its entirety is requested by each of the Opponents.

Additionally, each of the Opponents has made a subsidiary request for oral proceedings.

b) As regards the Proprietor

It is requested that the patent be maintained on the basis of the claims as mentioned in the observations of 08.04.1998 and filed with a letter dated 23.04.1998 and received on 24.04.1998.

Additionally, the Proprietor has made a subsidiary request for oral proceedings.

7). The list of documents as submitted by the Proprietor as Exhibit 1 of its observations of 08.04.1998, which contains all the documents cited by the parties, has been annexed to this communication. All the documents listed are the documents currently in file. In the following, the documents will be designated according to the references used by the Proprietor, the letter "**D**" being however replaced by the letter "**A**", to avoid any misunderstanding. When mentioned for the first time, each document will be additionally referred to by either the name of the first author mentioned therein and the publication date in the case of a scientific publication or the publication number in the case of a patent document.

8). Brief analysis of the comments made by Opponent 1

a). Objection as to lack of novelty

The subject-matter of claims 1-13, 15, 16, 18-20 and 22-24 has been alleged to lack novelty over document A12 (Hsiao et al, 1986).

Decisions T 114/86 and T 12/81 have been cited.

b). Objection as to lack of an inventive step

The claimed subject-matter has been alleged not to involve an inventive step:

- as regards claims 1, 3, 17-20 and 24: over document A12 (see page 19

[3rd full paragraph] of the notice of opposition).

- as regards claims 2, 4, 5, 8-10 and 16: over either of documents A12 and A2 (Hsiao et al, 1984), see page 30 [3rd full paragraph] of the notice of opposition.

- as regards claims 6 and 7: over document A3 (Weinstein et al. 1984), see the paragraph linking pages 20 and 21 of the notice of opposition.

- as regards claims 2, 4-10 and 16: over either of documents A12, A2 and A3 (see page 21 [2nd full paragraph] of the notice of opposition).

- as regards claims 11 to 15: over documents A12, A2, A3 and A17 (Johnson et al, 1987) taken into combination (see pages 21 [3rd to 5th paragraphs] and 22 [1st paragraph] of the notice of opposition).

- as regards claims 21, 22 and 23 over document A12 (see 2nd to 6th paragraphs of page 22 of the notice of opposition, respectively).

- as regards claims 25 and 26 over documents A12, A2, A3 and A17 (see page 23 of the notice of opposition).

c). Objection as to the presence of subject-matter which extends beyond the content of the application as originally filed

This objection appears not to have been anyway substantiated.

d). Additional remarks

Notwithstanding the mere comments made thereon on pages 2 and 3 of the notice of opposition, Opponent 1 has not pointed out how and to which extent documents D5-D11/O1 , i.e., documents A7 (Uehara et al, 1985), A4 (Kikkawa et al, 1984), A5 (Gooding et al, 1984), A18 (Johnson et al, 1987), A19 (Housey et al, 1987), A8 (Campert et al, 1985), and A27 (Housey et al,

1988), would be relevant as to the objections to lack of novelty and lack of inventive step.

On page 2 of the notice of opposition, Opponent 1 has alleged that the priority was not valid. Nevertheless, it has not been given any explanation in support of said allegation nor has it been explained to which extent such allegation would have an influence on the reasoning of Opponent 1.

9). Brief analysis of the comments made by Opponent 2

a). Objection as to lack of novelty

The subject-matter of claims 1 and 3 has been alleged to lack novelty over either document D1/O2, D2/O2, D3/O2 and D4/O2, i.e., documents A12, A25 (WO 89/03687), A9 (Drebin et al, 1985) and A26 (Housey et al, 1988); document A25 being taken as a document which is part of the state of the art as defined in Article 54(3) EPC).

The subject-matter of claims 2, 4, 7-9, 18, 19, 21, 22 and 24 has been alleged to lack novelty over document A12.

In contrast, it appears that the subject-matter of claims 6, 10-17, 20, 23, 25 and 26 has not been objected to.

b). Objection as to lack of an inventive step

The claimed subject-matter as a whole has been alleged not to involve an inventive step over documents D1/O2 + D3-D15/O2, i.e., documents A13 (Rosenthal et al, 1986), A9, A26, A20 (Weinstein et al, 1987), A17, A14 (Knopf et al, 1986), A19 (Housey et al, 1987), A10 (Angehrn, 1985), A22 (O'Brian et al, 1987), A23 (Debouck et al, 1987), A11 (Dotto et al, 1985), A2, and A15 (Shah et al, 1985), see 1st paragraph of section (2) on page 9 of the notice of opposition.

c). Objection as to the presence of subject-matter which extends beyond the content of the application as originally filed

It has been contended that claims 1, 2 and 25 would content subject-matter which extends beyond the content of the application as originally filed, for the reason that the method of the invention, when performed without using any control cells, would not be described in the application as originally filed.

10). Brief analysis of the comments made by Opponent 3

a). Objection as to lack of novelty

The subject-matter of certain claims has been alleged to lack novelty:

- claims 1, 3, 4, 5, 9, 18, 19 and 22 over either of documents D1/O3 and D2/O3, i.e., either of documents A24 (Di Fiore, 1987) and A12, see sections 1.1, 1.2, 1.3, 1.4, 1.7, 1.9, 1.10 and 1.11 of the notice of opposition.

- claims 7 and 8: over document D1/O3, i.e., document A24, see sections 1.5 and 1.6 of the notice of opposition.

- claims 15 and 23: over document D2/O3, i.e., document A12, see sections 1.8 and 1.12 of the notice of opposition.

In contrast, it appears that the subject-matter of claims 2, 6, 10-14, 16, 17, 20, 21, 24, 25 and 26 has not been objected to.

b). Objection as to lack of inventive step

The claimed subject-matter has been alleged not to involve an inventive step:

Section 10 (continued)

- as regards claims 1-3, 14 and 16-26: over either of documents D1/O3 and D2/O3, i.e., either of documents A24 and A12, taken into combination with document D3/O3, i.e., document A6 (Nishizuka, 1984), and/or document D4/O3, i.e., document A1 (Boreiko et al, 1980), see sections 2.1.7, 2.2, 2.3 and 2.10 of the notice of opposition,

  - as regards claims 4, 5 and 7-9: over either of documents D1/O3 and D2/O3, i.e., either of documents A24 and A12, see section 2.4 of the notice of opposition, 3rd full paragraph,

  - as regards claim 6: over document D3/O3, i.e., document A6, see section 2.5 of the notice of opposition,

  - as regards claim 10: over document D5/O3, i.e., document A16 (Darnell, 1986), see section 2.6 of the notice of opposition,

  - as regards claims 11 and 13: over documents D1/O3, D2/O3, D3/O3 and D4/O3, i.e., documents A24, A12, A6 and A1, see sections 2.7 and 2.9 of the notice of opposition,

  - as regards claim 12: over documents D2/O3 and D3/O3, i.e., documents A12 and A6, see section 2.8 of the notice of opposition, and

  - as regards claim 15: over document D1/O3, i.e., document A24, taken into combination with document D2/O3, i.e., document A12, see section 2.11 of the notice of opposition.

c). Objection as to insufficiency of disclosure

Opponent 3 has submitted that the requirements of Article 83 EPC are not met as regards the aspects of the invention dealing with the determination of a substance which is **an inhibitor** of a protein, see section 3.1 of the notice

of opposition.

11). Brief analysis of the observations filed by the Proprietor

a). Preliminary remarks

In his observations of 08.04.1998, the Proprietor mentioned that it is submitting an amended main [claim] request. Said claim request which was not enclosed with said observations was filed with a later submitted letter dated 23.04.98.

To avoid any ambiguity, it has to be pointed out that, nothwithstanding its designation, the said "*main* [claim] *request*" is the only claim request on file. It would be referred to by the Opposition Division in the rest of the procedure as "*the claim request of 23.04.1998*".

As explained by the Proprietor, the claim request of 23.04.1998 differs from the claims as granted only in that granted claim 1 has been amended in such a way that the term "***a*** *phenotypic characteristic*" (emphasis added) has been replaced by the term "***said*** *phenotypic characteristic*" (emphasis added).

The Proprietor has indicated where according to its views a support can be found for said amendment in both the application as originally filed and the patent as granted. It has also indicated that said amendment has been carried out to improve the clarity of the claim.

b). Observations made as regards the allegation that the patent as granted would contain subject-matter which extends beyond the content of the application as originally filed

The Proprietor has stated that Opponent I did not substantiate its allegation.

In reply to Opponent 2, the Proprietor has referred to statements made on pages 3 and 4 of the result of personal consultation enclosed with a

Section 11 (continued)

registered letter dated 08.12.1994 issued during the examination proceedings of application 89 902 686.8.

c). Observations made as regards the allegation that the priority would not have been validly claimed

The Proprietor has stated that Opponent I did not substantiate its allegation.

d). Observations made as regards the allegation that the claimed subject-matter is not new

As a preliminary remark the Proprietor has submitted that *the Opponents' allegations are to a great extent based on misconception and misinterpretation of the prior art and the patent and its claims*, more particularly as regards step (c) of claim 1.

Turning to the cited documents, the Proprietor made the following comments:

- As regards document A12:

  Document A12 *does not relate at all to activators or inhibitors within the meaning of the contested patent, namely substances which interact directly with a given target protein.* In this respect, the Proprietor has pointed out that *TPA directly interacts with (binds to) and activates PKC* [protein kinase C], *and NOT p21^{ras}* with the result that *TPA is not an activator of p21^{ras} under the definition of the contested patent.*

- As regards document A13:

  TGF-α exerts its effect extracellularly and is therefore not a POI [Protein Of Interest] *as recited in claim 1.*

Section 11 (continued)

Opponent 2 *is attempting to define an antibody which binds to TGF-α as an "inhibitor" of TGF-α. However, this is an incorrect interpretation of the concept of inhibitor as defined in the specification of the contested patent.*

Document A13 *has certainly NOT determined that TGF-β is an "activator" of TGF-α.*

- As regards document A25:

[T]*he example referred to by* Opponent 2 *merely demonstrates than an M-CSF molecule conjugated to a cellular toxin such as ricin will kill cells expressing an M-CSF receptor protein.*

Document A25 *does not at all mention activators or inhibitors or provides a method for their identification.*

- As regards document A9:

Document A9 *merely describes the reversion of the transformed phenotype of cells transformed with an oncogene to a non-transformed phenotype when these cells are treated with a monoclonal antibody against the gene product of said oncogene.*

The authors have NOT determined that anti p185 antibodies are "inhibitors" of p185 according to the definition of the invention.

- As regards document A26:

The Proprietor has stated that document A26 is an article published in August 1988, i.e., after the priority date of the contested patent and noted that, according to Opponent 2, said document would relate to a

Section 11 (continued)

lecture given in June 1987, i.e., before the priority date, in Toronto. The Proprietor added that anyway the actual content of said lecture does not disclose the claimed invention. In support of this submission, reference has been made to document A30 which is a Declaration of the inventor and the abstract of the lecture attached thereto.

- As regards document A27:

  Document A27, which was cited in document A26 as being in press, was submitted on October 13, 1987, i.e., four months after the lecture in Toronto and, therefore, accepted only after said lecture.

- As regards document A24:

  The authors have not defined or characterized a responsive change in a phenotypic characteristic and demonstrated that said phenotypic characteristic is responsive to inhibitors or activators of a given target protein (in this case the EGFR).

  The experiments performed by these authors do not result in findings which determine that EGF is an activator of the EGFR.

  The authors do not even discuss the possibilty that such a system could be used to identify such substances.

The Proprietor has come to the conclusion that none of the said documents anticipate the claimed subject-matter.

Section 11 (continued)

e). Observations made as regards the allegation that the claimed subject-matter would not involve an inventive step

- Closest prior art:

  The Proprietor has considered that the closest prior art could be seen in documents which relate to conventional methods for the identification of activators or inhibitors of a POI that were employed before the present invention was made. As such a document has not been cited by the Opponents, the Proprietor has proposed that document A12 be seen as the closest prior art.

- Technical problem:

  The Proprietor has defined the technical problem underlying the invention as the provision of *a rapid and easy method for the detection of specific chemical activators and inhibitors of a protein which interact directly with the protein and modulate its cellular activity(ies) in a useful manner.*

- Person skilled in the art:

  The Proprietor has contended that the person skilled in the art can be *a cautious bench molecular biologist or a team of such persons being familiar with the principles of recombinant DNA technology and expression of foreign proteins in cultured cells* and regarded it as *inadmissible to attribute the knowledge provided by the contested patent to the skilled person in the art in the analysis of inventive step.*

  Decision T455/91 has been cited.

Section 11 (continued)

- Observations made regardings the allegations of the Opponents:

(1) Of Opponent 1

The Proprietor has stated that the simple assay system as described in document A12 may be useful for identifying new tumor promoters and that substances such as TPA and teleocidin whose activities include activation of several PKC isoforms might be identifiable with this system.

The Proprietor has added that *under no circumstances would this assay system as disclosed be able to provide any of the susbstantive target protein-specific pharmacological information which is the hallmark of the cell-based assay system of Patentee's invention.*

(2) Of Opponent 2

The Proprietor made the following comments:

- Cells have been used in screening processes in the past but *no such work at any time prior to the contested patent has ever taught that it is possible to determine whether a substance is an inhibitor or an activator of a given protein-of-interest (POI or "target" protein), or to otherwise pharmacologically characterize a substance with respect to its effects upon an individual target potein, using a responsive change in a phenotypic characteristic of a cell.*

- Regarding documents A2, A9, A10, A11, A14, A15, A17, A18 and A19, *none of them are able to determine whether or not any substance tested is an inhibitor or an activator of any*

Section 11 (continued)

given target protein based upon the changes in a cellular characteristic, if any, of a cell treated with a substance.

- A method of determing whether a substance may be an inhibitor of PKC with or through the use of a cell-based - assay is not disclosed or suggested in document A20, notwithstanding the presence of a speculative sentence on page 181 which reads "*the development of specific inhibitors of PKC could provide a powerful new approach to the control of cell proliferation*".

- Whereas the subject-matter of document A5 is concerned with the mechanism of action of protein kinase C and its role in cancer, nowhere in this reference is the concept of a cell-based assay system discussed.

- As regards document A10, whereas Opponent 2 is suggesting that cephalosporinase is the POI, no conclusion regarding the ability of carumonam to inhibit or activate this enzyme may be determined.

- The findings on which rely documents A13 and A14 have nothing to do with the issue of determining whether a substance is an activator or an inhibitor of a given target protein by using a responsive change in a phenotypic characteristic of a cell.

- As regards document A15, whereas this paper deals with the powerful herbicide glyphosphate, which has been shown to inhibit the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase in a wide variety of plant cells which results in cell death, this work provides no insight as to how a cell

Section 11 (continued)

may be used to determine whether a substance is an inhibitor or an activator of a given target protein.

(3) Of Opponent 3

As regards document A6, while agreeing that *the findings which resulted in determining that TPA, teleocidin, aplysiatoxin and mezerein are activators of certain PKC isoforms were determined using cell-free enzyme assays containing one or more isozymes of partially purified protein kinase C*, the Proprietor has stressed the point that none of said determinations was made using the cell-based assays of the invention.

(4) The Proprietor has reached the conclusion that none of the documents cited by the Opponents, *individually of taken together, conceives, develops or reduces to practice a cell-based assay system whereby a chemical substance can be determined to be an activator or an inhibitor of a given target protein and which interacts directly with said target protein.*

f). Observations made as regards the allegation that the disclosure would not be sufficiently clear and complete within the meaning of Article 83 EPC

The Proprietor has made the following comments:

- The description of the patent deals directly with the use of inhibitors in cell-based assay systems, page 13 being referred to.

- The examples of the patent deal with the screening of a potent inhibitor of the c-H-ras oncogene product.

- Experimental results of testing inhibitors are provided on page 15 of the patent.

- Even if there were no examples in the patent demonstrating the screening of inhibitors of a POI, the objection of insufficiency of the disclosure would be irrelevant as it is not necessary to provide examples at all, let alone for all embodiments claimed.

12). Provisional opinion of the Opposition Division on the basis of the claim request of 23.04.1998

a). On the admissibility of the amendment contained in the claim request of 23.04.1998

According to Rule 57a EPC only amendments which are occasionned by grounds of opposition specified in Article 100 EPC can be admitted.

It may be considered that claim 1 as granted has been amended to reject the allegation made by Opponent 1 as regards lack of novelty (see the comments made with respect to document A12 on pages 10 to 15 of the Proprietor's letter of 08.01.1998 [see especially sections 1.1 and 1.7]).

Therefore, it can be considered that said amendment appears to meet the requirements as et forth in Rule 57a EPC. Furthermore, it can also be considered that it is allowable under both Articles 123(2) (see page 11 [lines 12-17] of application WO 89/07654) and 123(3) EPC (as the amendment has resulted in a limitation of the subject-matter as claimed in the patent).

Nevertheless, claim 1 as amended does not appears to meet the requirements of Article 84 EPC. Insofar as the "*responsive change in a phenotypic characteristic*" referred to in the preamble is the "*phenotypic response*" referred to in step (a), it would appear that step (c) aims at determining whether said cell exhibits not "*a responsive change in said phenotypic characteristic*" [as presently formulated] but "*a responsive*

Section 12 (continued)

*change in said phenotypic **response***", which formulation allows to clearly distinguish the change which is responsive to the protein (as mentioned in the preamble) with the change which is responsive to the "substance" tested,

see further the following phrase in the forth sentence of Chapter E on page 9 of the Proprietor's letter of 08.04.1998, which reads "the person skilled in the art does not look to see if something (anything) happens, but whether or not *a change has occured in **said** phenotypic response*".

b). On the allegation that the patent contains subject-matter which extends beyond the content of the application as originally filed

The Opposition Division confirms the opinion expressed in item 7 of the result of consultation issued on 08.12.1994 during the examination proceedings of application 89 902 686.8 and considers that the patent as granted and as presently amended, the claimed method being performed without any control cells, meets the requirements as set forth in Article 123(2) EPC. In this respect, the attention of Opponents 1 and 2 is especially drawn to page 13, lines 1-3 of WO 89/07654.

c). On the allegation that the patent does not disclose the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art

Taking into consideration the comments made on the screening for a potent inhibitor of the c-H-ras oncogene product in Example 2 (see page 13 of the patent), and the mention made therein that "*screening for a potent p21 inhibitor could then be performed as described in Example 1*", and as Opponent 3 has not provided any experimental proof that a person skilled in the art would not have been in a position to screen for a potent p21 inhibitor on the basis of the instructions contained in said Examples, the Opposition Division is of the opinion that it has to be considered that the requirements

Section 12 (continued)

as set forth in Article 83 EPC are met.

d). On the allegation that the claimed invention is not new or does not involve an inventive step

* The following comments are made on the basis of the claim request of 23.04.19978 assuming that claim 1 would meet the requirements of article 84 EPC.

d1). Preliminary remark

The Proprietor has stressed the point that in the light of the description as originally filed ① the phenotypic characteristic is determined by **the activity** of the target protein [= the protein of interest] in a cell, ② said phenotypic characteristic **is responsive to** inhibitors or activators of said protein, ③ activators or inhibitors within the meaning of the patent are substances which **modulate the activity** of said protein , i.e., which **interacts directly** therewith (see, with respect to ①: page 10, third paragraph, with respect to ②: page 13, second paragraph, first sentence, and page 11, first paragraph, and with respect to ③: page 11, third paragraph, page 12, second paragraph, page 30, second sentence of the summary, and page 31, second sentence, respectively, of the Proprietor's letter of 08.04.1998).

The Opposition Division agrees with this analysis.

Section 12 (continued)

d2). Comments as regards the allegation that the claimed invention is not novel

(1) Brief analysis of the content of documents A9, A12, A13, A24, A25 and A26)

A9: Describes that exposure of neu-oncogne-transformed NIH 3T3 cells to monoclonal antibodies reactive with the neu gene product, p185, results in the rapid and reversible loss of both cell-surface and total cellular p185.

The Opposition Division notes that such antibody-mediated down-modulation of cell surface p185 does not result in inhibition of a given target protein as defined in the patent. Furthermore, said antibodies appear not to enter the cell; therefore, A9 dose not describe the screening of any activator or inhibitor within the meaning of the patent.

A12: As explained on page 1950, left-column, the tumor promoters referred to therein, i.e., TPA and teleocidin, appear to neither interact nor directly activate the $p21^{ras}$ protein which is the protein that is overproduced in the cells used in the assay described therein. Therefore, the assay of document A12 fails to identify activators [or inhibitors] within the meaning of the patent.

A13: As the antibodies with bind to TGF-α referred to therein appear not to enter the cell and as TGF-α appears to exert its effects through receptors on the cell (see A13, page 304, second paragraph of the section entilted

Section 12 (continued)

"*Discussion*", last sentence), i.e., is not a protein of interest as recited in claim 1 [which refers to "*a protein whose presence **in** a cell*"], said antibodies cannot be considered as inhibitors [of TGF-α] within the meaning of the patent. Similarly, as the potentiation of the effects of TGF-α by TGF-β does not appear to be the result of a direct interaction between the two molecules but may be attributed to the ability of TGF-α to induce an increase in the number of EGF receptors on the cell surface (see A13, page 302, right-hand column), nor can TGF-β be considered as an activator [of TGF-α] within the meaning of the patent.

A24: Said document demonstrates the reconstitution of functional EGF receptors in mutant NR6 cells that have lost the ability to express such receptors by transfection of a vector encoding the EGF receptor (EGFR) molecule to induce overexpression of the same in said cells. Furthermore, it shows that introduction of said vector into NIH 3T3 cells, which normally respond to EGF but display low numbers of receptors, led to no gross alterations in cell growth properties in the absence of EGF, with the further observation that the addition of EGF alters the growth properties of the cells.

The Opposition Division agrees with the Proprietor's opinion (see page 20 of his letter of 08.04.1998) that A24 as such does not describe a method of determining whether a substance is an inhibitor or an activator of a protein as defined in claim 1 of the patent. It is clear that the authors of A24 have not defined in

Section 12 (continued)

advance, i.e., before carrying out their experiments, that the presence of EGFR in NIH3T3 or NR6 cells would induce a change in a phenotypic characteristic and have not carried out the experiments with a view to assessing whether upon EGF addition a change in said phenotypic response was to be observed.

A25: * is part of the state of the art as defined in Article 54(3) EPC

It cannot be seen from Example 3 (see A25, page 14) how an M-CSF molecule conjugated to an acellular toxin which target the toxin to M-CSF receptor - expressing cells [with the result that the toxin penetrates the cell and kills it] could be regarded as an activator or an inhibitor within the meaning of the patent.

A26: As pointed out by the Proprietor on page 19 of its letter dated 08.04.1998, said document was published in August 1988, i.e., after the priority date (10.02.1988) of the patent; as such, as it has not been established that the patent would not have been entitled to its priority date, it has to be considered that A26 is not part of the relevant state of the art to be taken into account.

(2) Conclusion

The Opposition Division is of the opinion that none of the afore-mentioned documents discloses a method as defined in either claims 1 and 3 of the patent and that, therefore, the claimed invention as a whole can be regarded as new over the cited prior art documents.

Section 12 (continued)

d3). Comments as regards the allegation that the claimed invention does not involve an inventive step

(1) Analysis of the content of documents A1, A3, A4, A5, A6, A7, A8, A10, A11, A14, A15, A16, A17, A18, A19, A20, A21, A22 and A23

A1: Reports on studies examining the effects of TPA on numerous properties of C3H/10T1/2 Cl 8 cells, several of which are known to be altered in oncogenic transformation.

A2: Reports on experiments which show that oncogene-induced transformation of C3H 10T1/2 cells is enhanced by tumor promoters.

A3: Reviews recent studies on the biochemical effects of tumor initiators and promoters and then considers how they might contribute to the phenotype of fully evolved tumors.

A4: Reports on experiments which have been carried out to explore the roles of protein kinase C which is considered as being most likely a receptive protein of tumor promoters to activate cellular functions and proliferation.

A5: Reports that a panel of monoclonal anti-T-antigen antibodies has been employed to investigate the expression of individual T-antigenic determinants on the cell surface. The results have been then compared with information available on the specificity of SV40-immune cytotoxic T cells.

Section 12 (continued)

A6: The purpose of this review was to summarize the evidence relating to the activation of protein kinase C and to the importance of that activation for subsequent cellular responses. TPA, teleocidin, *Aplysia* toxin and mezerein are listed as activators of PKC (see page 695, right-hand column).

A7: The authors report that during the course of screening of agents active in converting the transformed morphology of Rous sarcoma virus-infected rat kidney cells to the normal morphology they have identified herbimycin, a known antibiotic produced by a strain of *Streptomyces*, as an active substance which was found to be an inhibitor of $pr60^{src}$-associated protein kinase in the cells.

A8: Reports on an investigation of the hormonal regulation of the bovine prolactin promoter in rat pituitary tumor cells.

A10: Reports on the antibacterial properties of *carumomam*, a sulfonated monocyclic β-lactam antibiotic.

A11: Show that, like initiated cells, primary rat embryo fibroblasts containing a *ras* but not a *myc* oncogene, are strongly and specifically stimulated to grow by tumor promoters.

A13: Reports on experiments which show that expression in rat fibroblasts of a human transforming growth factor-α cDNA results in transformation.

A14: Reports on the discovery of a family of protein-kinase-C-related polypeptides and the implications thereof.

Section 12 (continued)

A15: Report that a chimeric ESSP synthase gene was constructed with the use of cauliflower mosaic virus 35S promoter to attain high level of expression of EPSP synthase and introduced into petunia cells. Transformed petunia cells as well as regenerated transgenic plant were tolerant to glycophosphate which is a potent inhibitor of ESPS synthase in higher plants.

A16: Reports on the use of flow cytometry to sort cells.

A17: In this paper, the authors describe the isolation and characterisation of a cDNA clone corresponding to a gene whose expression is increased in C3H 10T1/2 cells in response to TPA, a cDNA representing a gene whose abundance is decreased by TPA, and evidence for the role of PKC in their regulation.

A18: Provides evidence that the induction of TPA-S1-RNA by TPA is mediated by PKC and discusses the relevance of studies of PKC and signal transduction to the development of short-term assays for tumor promoters.

A19: Reports on the isolation of a cDNA clone encoding the carboxyl terminaus of rat brain PKC and describes a closely related yet distinct cDNA clome that appears to belong to a novel PKC-related multigene family.

A20: Reports on the mechanism of action of protein kinase C and the isolation of molecular clones encoding the enzyme.

A21: Reports on the expression and processing of the AIDS virus reverse transcriptase in *Escherichia coli.*

Section 12 (continued)

A22: Reports on experiments whose results suggest that synthetic peptide substrates of PKC provide important tools for further probing the mechanism of action of tumor promoters at the enzymatic level and also for providing clues to cellular target proteins that might be involved in tumor promotion.

A23: Reports on expression of the human immunodeficiency virus protease gene product in *Escherichia coli* which was shown to autocatalyze its maturation from a larger precursor.

(2) Comments about the reasons put forward by Opponent 1

According to Opponent 1, in view of document A12 taken alone, the subject-matter of claims 1, 3, 17 to 20 and 24 would not involve an inventive step.

Insofar as it has been acknowledged that document A12 failed to identify activators and inhibitors within the meaning of the patent (see item 9(d)(d2)(1) above), it has to be concluded that said document on its own cannot be relevant when assessing whether the subject-matter of any claims involves an inventive step.

Furthermore, as none of documents A2, A3 and A17 (see comments in item (1) above) is directed to the identification of activators or inhibitors as defined in the patent, they could not deprive any aspect of the claimed subject-matter from involving an inventive step, even if, as proposed by Opponent 1, taken in combination with document A12.

Section 12 (continued)

(3) Comments about the reasons put forward by Opponent 2

The Opposition Division which is quite in agreement with the opinion expressed by the Proprietor on pages 26 to 29 of his letter of 08.04.1998 especially considers that:

- The documents cited by Opponent 2 which are part of the state of the art as defined in Article 54(2) EPC, i.e., documents A2, A9, A10, A11, A13, A14, A 15, A17, A19, A20, A21, A22 and A23, while providing cells which could have been used in screening processes, are silent about the determination of whether or not any substance tested is an inhibitor or a activator of any given target protein based upon the changes in a cellular characteristic of a cell treated with said subtance.

- Document A20 is certainly interested with the identification of inhibitors of PKC some of which could meet the definition therefor given in the patent (see A20, pages 180-181) but it does not provide any precise guidelines as how to technically proceed therefor.

- As regards document A10, there can be no determination made as to what target protein(s) are involved in carumomam's antibacterial activity .

- Documents A13 and A14 have clearly nothing to do with the issue of determining whether a substance is an activator or an inhibitor of a given target protein by using a responsive change in a phenotypic characteristic of a cell (see the comments made as regards these documents in iem (1) above).

Section 12 (continued)

- Whereas document A15 describes a cell-line which was shown to overproduce EPSP synthase messenger RNA and, therefore, might be of interest for carrying out a method as defined in the claims in the patent [with a view to identifying activators or inhibitors of EPSP synthetase], said document appears to provide no insight as to how a cell may be used to determine whether a substance is an inhibitor or an activator of a given target protein.

Therefore, the Opposition Division is of the opinion that none of the documents cited by Opponent 2, when taken alone or in combination would deprive the whole claimed subject-matter from involving an inventive step.

(4) Comments about the reasons put forward by Opponent 3

Documents A1, A6, A12, A16 and A24 have been cited by Opponent 3.

As regards documents A12 and A24 it is referred to the comments already made thereon at item 9(d)(d2)(1).

In view of the content of each of documents A1 and A16 (see the comments made above at item (1) thereon), and as Opponent 3 has not provided any detailed explanation as to the relevance of document A1 (see section 2.10 of the notice of oppostion), it does appear how any combination of said documents with document A12 which, as already emphasized, fails to identify activators or inhibitors within the meaning of the patent, could deprive the subject-matter of any of the claims from involving an inventive step.

Furthermore, as none of documents A1 and A16 would have incited a person skilled in the art to use the cell system of document A24 in a screening assay relying on a procedure as defined in the method of any of claims 1 and 3, the Opposition Division further considers that documents A1 and A16, alone or in combination, when taken together with document A24 would deprive the whole claimed subject-matter from involving an inventive step.

As to document A6, the Opposition Division is in agreement with the opinion expressed by the Proprietor page 29 of his letter of 08.04.1998 and considers that it is clearly irrelevant.

Therefore, the Opposition Division is of the opinion that none of the documents cited by Opponent 3, when taken alone or in combination, would deprive the whole claimed subject-matter from involving an inventive step.

e) Conclusion

The Opposition Division is of the provisional opinion that the present grounds for opposition would not prejudice maintenance of the patent in its present amended form provided that claim 1 be reformulated in such a way that the requirements of Article 84 EPC be met.

If claim 1 were not reformulated, it would apear that the patent should be revoked for the reason that the patent does not meet all the requirements of the Convention (see Article 102(3) EPC).

13). To comply with the auxiliary requests of the parties, oral proceedings are summoned by the end of which it is intended that a final decision will be taken on the present opposition.

14). The parties are advised that written submissions in preparation to oral proceedings should be made not later than two months before the date on which the oral proceedings will take place, see further Rule 71a(1) EPC.

T. Mennessier H. Hüssel M. Götz

LIST OF DOCUMENTS CITED IN THE PROCEEDINGS

Doc.	Cited by OI Hoechst AG	OII Boehringer Mannheim	OIII Boehringer Ingelheim	Reference
D1			D4	Boreiko, Cancer Research 40 (1980), 4709-4716
D2	D2	D14		Hsiao, Science 226 (1984), 552-555
D3	D3			Weinstein, Cancer Cells 1 (1984), 229-237
D4	D6			Kikkawa, Cancer Cells 1 (1984), 239-244
D5	D7			Gooding, Cancer Cells 1 (1984), 263-269
D6			D3	Nishizuka, Nature 308 (1984), 693-698
D7	D5			Uehara, Jpn. J. Cancer Res. 76 (1985), 672-675
D8	D10			Campo, J. Biol. Chem. 260 (1985), 12246-12251
D9		D3		Drebin, Cell 41 (1985), 695-706
D10		D9		Angehrn, Chemotherapy 31 (1985), 440-450
D11		D13		Dotto, Nature 318 (1985), 472-475
D12	D1		D2	Hsiao, Mol. Cell. Biol. 6 (1986), 1943-1950
D13		D1		Rosenthal, Cell 46 (1986), 301-309
D14		D7		Knopf, Cell 46 (1986), 491-502
D15		D15		Shah, Science 233 (1986), 478-481
D16			D5	J. E. Darnell, Mol. Cell. Biol. Scientific American Books, Inc. (1986)

Doc.	Cited by OI Hoechst AG	OII Boehringer Mannheim	OIII Boehringer Ingelheim	Reference
D17	D4	D6		Johnson, C. Mol. Cell. Biol. 7 (1987), 2821-2829
D18	D8			Johnson, Environmental Health Perspectives 76 (1987), 89-95
D19	D9	D8		Housey, Proc. Natl. Acad. Sci. USA 84 (1987), 1065-1069
D20		D5		Weinstein, Cancer Res. 39 (1987), 173-183
D21		D10		Farmerie, Science 236 (1987), 305-308
D22		D11		O'Brian, Cancer Cells 3 (1987), 359-363
D23		D12		Debouck, Proc. Natl. Acad. Sci. USA 84 (1987), 8903-8906
D24			D1	Di Fiore, Cell 51 (1987), 1063-1070
D25		D2		WO 89/03687 (1989)[1]
EP-B1 0 403 506				February 10, 1988 P.D.
D26		D4		Housey, Adv. in Exp. Med. Biol. 234 (1988), 127-140
D27	D11			Housey, Cell 52 (1988), 343-354
EP-B1 0 403 506				February 9, 1988 F.D.
D28				Alberts, Molecular Biology of the Cell, 3rd ed. (1994), Garland Pub., NY USA, 1264-1265
D29				Chapter 2 of "The Pharmacological Basis of Therpeutics", Goodman & Gilman (9th Ed. 1996)
D30				Declaration of Dr. Housey

[1] Priority date of October 23, 1987

P.D. Priority date

F.D. Filing date

Claims

1. A method of determining whether a substance is an inhibitor or an activator of a protein whose presence in a cell evokes a responsive change in a phenotypic characteristic other than the level of said protein in said cell per se, which comprises:

   (a) providing a cell which overproduces said protein and exhibits said phenotypic response to the protein;

   (b) incubating said cell with said substance; and

   (c) determining whether said cell exhibits a responsive change in said phenotypic characteristic.

2. The method of claim 1, wherein said responsive change is a graded cellular response to said substance.

3. A method of determining whether a substance is an inhibitor or activator of a protein whose presence in a cell evokes a responsive change in a phenotypic characteristic other than the level of said protein in said cell per se, which comprises:

   (a) providing a first cell which overproduces said protein and exhibits said phenotypic response to the protein;

   (b) providing a second cell which produces the protein at a lower level than the first cell, or does not produce the protein at all, and which exhibits said phenotypic response to the protein to a lesser degree or not at all;

   (c) incubating the first and second cell with the substance; and

   (d) comparing the phenotypic response of the first cell to the substance with the phenotypic response of the second cell to the substance.

4. The method of any one of claims 1 to 3, wherein the response is one observable with the naked eye.

5. The method of any one of claims 1 to 4, wherein the response is a change in a cultural or morphological characteristic of the cell.

6. The method of claim 5, wherein the response is a change in the differentiation state of the cell.

7. The method of any one of claims 1 to 4, wherein the response is a change in the ability of the cell line to grow in an anchorage-independent fashion.

8. The method of any one of claims 1 to 4, wherein the response is a change in the ability of the cell line to grow in soft agar.

9. The method of any one of claims 1 to 4, wherein the response is a change in foci formation in cell culture.

10. The method of any one of claims 1 to 4, wherein the response is a change in the ability of the cell to take up a selected stain.

11. The method of any one of claims 1 to 10 in which the protein is an enzyme.

12. The method of claim 11, wherein increased activity of the enzyme is correlated with increased tumorigenesis.

13. The method of claim 12 in which the enzyme is a protein kinase C enzyme or a fragment, domain or subunit of a receptor which has protein kinase C activity.

14. The method of claim 12, wherein the enzyme is ornithine decarboxylase.

15. The method of any one of claims 1 to 10 in which the protein is the expression product of an oncogene.

16. The method of any one of claims 1 to 10, wherein the response is a change in an antigenic characteristic of the cell.

Exhibit E

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Application No. / Patent No.	Ref.	Date
89 902 686.8-2116 / 0403506 /	A 205 EP	26. 04. 00
Proprietor: HOUSEY, Gerard M.		

Interlocutory decision in Opposition proceedings (Article 106(3) EPC)

The Opposition Division - at the oral proceedings dated 09.12.1999 - has decided:

Account being taken of the amendments made by the patent proprietor during the opposition proceedings, the patent and the invention to which it relates are found to meet the requirements of the Convention.

The reasons for the decision are enclosed.

Documents for the maintenance of the patent as amended:

Text for the Contracting States:
AT BE CH LI DE FR GB IT LU NL SE

Description, pages:

1-3,6,7,9-18	of the patent specification	
4,5,8	during oral proceeding on	09.12.1999

Claims, No.:

1-26	during oral proceeding on	09.12.1999

Drawings, No.:

1,1B-1C,2-7	of the patent specification

Registered letter with advice of delivery

Possibility of appeal
This decision is open to appeal. Attention is drawn to the attached text of Articles 106 to 108 EPC.

Opposition Division:

Chairman:	GOETZ M E
2nd Examiner:	HOESEL H R
1st Examiner:	MENNESSIER T J H

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Enclosure(s): 25 page(s) reasons for the decision (Form 2916)
Wording of Articles 106 - 108 (Form 2019)
Documents relating to the amended text
[ ] Minutes of oral proceedings
Annexes 1 (Form 2916), 2 (Form 2916) and 3

to EPO postal service: 19.04.2000

Registered l tter with advice of delivery
EPO Form 2327 02.99CSX

Facts and submissions

I. European patent No. 0 403 506 B1 is based upon European patent application No. 89 902 686.8 claiming a priority of 10.02.1988. Said application is derived from an international application filed under the PCT on 09.02.1989 and published under the international publication number WO 89/07654. The title of the patent is "*Method of screening for protein inhibitors and activators*".

II. The Proprietor of the patent is: **Mr. Housey Gerard M.**
New York, NY 10032
United States of America

III. The Opponents are:

Opponent 1: **Hoechst AG**
D-65926 Frankfurt am Main

Opponent 2: **Roche Diagnostics GmbH**
Sandhofer Straße 116
D-68305 Mannheim

Opponent 3: **Boehringer Ingelheim GmbH**
D-55216 Ingelheim am Rhein

IV. The three opponents request that the patent be revoked in its entirety whereas the Proprietor requests that the patent be maintained in an amended form on the basis of the set of claims as filed at the oral proceedings held on 09.12.1999 as amended during the said proceedings.

V. The current documents on which the decision is to be based are documents A1 to A41 as listed in Annex 1 to the present grounds for the decision.

Brief overview of the written phase

VI. In its notice of opposition each of the opponents alleged that the subject-matter of

the claims as granted was neither novel nor inventive, according to grounds of opposition referred to in Article 100(a) EPC). Additionally, Opponents 1 and 2 considered that the said subject-matter extended beyond the content of the application as originally filed, according to the ground of opposition referred to in Article 100(c) EPC, while Opponent 3 argued that the patent did not disclose the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art, according to the ground of opposition referred to in Article 100(b) EPC). Furthermore, Opponent 1 submitted that the priority had not been validly claimed but it did not substantiate that submission.

VII. In reply to the notices of opposition the Proprietor requested that the patent be maintained in an amended form on the basis of a set of claims filed with a letter dated 23.04.1998. That set of claims (referred to hereafter as the set of claims of 23.04.1998) differed from the claims as granted only in that granted claim 1 (see Annex 2 to the present grounds for the decision) has been amended in such a way that the term "***a*** *phenotypic characteristic*" has been replaced by the term "***said*** *phenotypic characteristic*" (see Annex 2 to the present grounds for decision).

VIII. With an official communication dated 21.04.1999 the Opposition Division expressed the following provisional opinions:

a) The amendment contained in the set of claims of 23.04.1998 met the requirements of Rule 57a EPC and was allowable under Articles 123(2) and (3) EPC. In contrast, it did not meet the requirements of Article 84 EPC. In this respect it was stated that '[I]nsofar as the "*responsive change in a phenotypic characteristic*" referred to in the preamble is the "*phenotypic response*" referred to in step (a), it would appear that step (c) aims at determining whether said cell exhibits not "*a responsive change in said phenotypic characteristic*" [as presently formulated] but "*a responsive change in said phenotypic* ***response***", which formulation allows to clearly distinguish the change which is responsive to the protein (as mentioned in the preamble) with the change which is responsive to the "substance" tested'.

b) It was considered that the patent as amended with the letter of 23.04.1998, the claimed method being performed without any control cells, met the requirements as set forth in Article 123(2) EPC. In this respect, the attention of Opponents 1 and 2 was especially drawn to page 13, lines 1-3 of WO 89/07654.

c) Taking into consideration the comments made on the screening for a potent inhibitor of the c-H-ras oncogene product in Example 2 (see page 13 of the patent), and the mention made therein that "*screening for a potent p21 inhibitor could then be performed as described in Example 1*", and as Opponent 3 had not provided any experimental proof that a person skilled in the art would not have been in a position to screen for a potent p21 inhibitor on the basis of the instructions contained in said Examples, the Opposition Division considered that the requirements as set forth in Article 83 EPC were met.

d) None of the various documents cited by the opponents, namely documents A9, A12, A13, A24, A25 and A26 disclosed a method as defined in either of claims 1 and 3 of 23.04.1998 and that, therefore, the claimed invention as a whole could be regarded as new.

e) None of the documents cited by the opponents (**A2**, A3, **A12** and A17 were cited by Opponent 1; **A2**, A9, A10, A11, A13, A14, A15, A17, A19, A20, A21, A22 and A23 were cited by Opponent 2; and A1, A6, **A12**, A16 and A24 were cited by Opponent 3), when taken alone or in combination, would deprive the whole claimed subject-matter from involving an inventive step.

IX. Finally, the Opposition Division provisionally concluded that as claim 1 contravened Article 84 EPC, the patent as amended with the set of claims of 23.04.1998 did not meet all the requirements of the convention. To comply with auxiliary requests therefor made by the parties, oral proceedings were summoned.

X. In a letter dated 07.10.1999, Opponent 1 substantiated its previous allegation that the claims of 23.04.1998 were not entitled to the priority date, and confirmed its

further previous allegations that (i) the invention was not sufficiently disclosed (only a brief reference to the notice of opposition was made), and (ii) the claimed invention was not novel in view of any of documents A12, A26 and A27.

XI. In a letter dated 07.10.1999, Opponent 2 alleged that (i) the subject-matter of the set of claims of 24.03.1998 was not novel in view of any of nine further documents referred to as documents A31, A32, A33, A34, A35, A36, A37, A38 and A39, and (ii), whereas its opposition was not based on this ground, that the invention was not sufficiently disclosed.

XII. In a letter dated 15.11.1999 and filed after the final date fixed according to Rule 71a(1) EPC for making written submissions in preparation for the oral proceedings, Opponent 3 alleged that the subject-matter of the set of claims of 23.04.1998 was not novel in view of either documents A42 and A43 cited for the first time in the said letter.

XIII. With a letter dated 08.10.1999, the Proprietor stated that he maintained the set of claims of 24.03.1998, representing its main claim request, and filed an auxiliary request differing from the main claim request in that claim 1 of said request had been amended in order that the deficiency under Article 84 EPC identified by the Opposition Division in its official communication of 21.04.1999 be removed.

Oral Proceedings held on 09.12.1999

XIV. The Proprietor filed a further claim request and requested that the patent be maintained in an amended form on the basis of the said claim request. The late filed documents A42 and 43 were not introduced into the procedure as being no more relevant than the documents filed earlier. Claim 3 of the claim request was amended. The opponents requested that the patent be revoked in its entirety. Documents A2, A7-A9, A11-13, A18, A31 and A34-A39 were cited by the opponents against novelty of the claims. The opponents also considered that the claimed invention did not involve an inventive step. In this respect, documents A2, A7 and A12 were cited. Objections under Articles 83, 84 and 123 EPC were also raised by the opponents. Finally, account being taken of the amendments made during the oral proceedings by the Proprietor, the patent and the invention to

which it relates were found by the Opposition Division to meet the requirements of the EPC.

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Reasons for the decision

1. The decision is taken as regards the set of claims filed and amended during the oral proceedings (see Annex 3 to the present grounds of opposition).

1a. There are 26 claims. Claims 1, 3, 24 and 25 are independent. Claim 2, claims 4-23 and claim 26 are dependent on claim 1, claim 3 and 25 respectively.

1b. Each of claim 1 and 3 is directed to **a method of determining whether a substance is an inhibitor or an activator of a protein**. The claimed methods require testing the substance on an overproducing cell line and involve a comparison of the phenotypic response of the said cell line to the substance with the phenotypic response of another cell line to the substance.

1c. Claim 24 is directed to **the use of a cell line** which overproduces a selected protein in a method according any one of claims 1 to 22. Claim 25 is directed to **a test kit** for carrying out the method of any one of claims 1 to 23.

2. Account has been taken of all the documents filed by the parties before the final date for making written submissions in preparation for the oral proceedings, i.e., documents A1 to A41. Documents A42 and A43 filed after the said date have not been introduced into the procedure because the Opposition Division has considered that they are not more relevant than the documents cited earlier. In this respect, it has been noted than none of them discloses a screening assay.

Admissibility of the amendments contained in the claims

3. The claims differ from the claims as granted in that:

   a) in each of claims 1 and 3, the word "*line*" has been added in the characterising part after each of the words "*cell*", the term "cell" in the last line of claim 3 having been amended into "*cell line*" after distribution of the set of claims by the Proprietor to the opponents and the Opposition Division.

   b) in claim 1 step (c) has been amended to read "***comparing the phenotypic response of said cell line of (a) to the substance with the phenotypic response of a control cell line to the substance to*** *determine whether said cell line of (a) exhibits a responsive change in said phenotypic response*",

      # the sentence in bold characters has been added to the claim as granted

      # the underlined word "*said*" before the term "*phenotypic response*" at the end of the statement replaces the word "*a*" used in the claim as granted

      # the term "*phenotypic response*" at the end of the statement replaces the term "*phenotypic characteristic*" used in the claim as granted

   c) In claims 24, 25 and 26, the word "*line*" has been added after each of the words "*cell*".

4. A support in the description as originally filed (see application WO89/07654) can be found in different locations and, more particularly:

   a) as regards the use in claim 1 of the word "*said*" instead of the word "*a*": on page 9, lines 15 to 18 and on page 40, lines 20 to 25,

   b) as regards the use in claims 1, 3, 24, 25 and 26 of the term "*cell line*" instead of the the word "cell": on page 4, line 3,

c) as regards the use in claim 1 of the term "*phenopypic response*" instead of the term "*phenotypic characteristic*": (i) on page 11, lines 12 to 17 in combination with page 13, second full paragraph, and (ii) in original claim 21,

d) as regards the additional statement in part (c) of claim 1: in original claim 1 (see feature (d)) and on page 5, lines 18-23.

5. Replacement in claim 1 of the term "*phenotypic characteristic*" by the term "*phenopypic response*" has been carried out in reply to an observation of the Opposition Division which had considered that the earlier claim request filed with the letter of 08.04.1998 did not meet the requirements of Article 84 EPC (see point 12(a) of the official communication dated 21.04.1999). All the other amendments appear to have been occasioned by grounds for opposition specified in Article 100 EPC and meet the requirements of Rule 57(a) EPC.

6. Opponents have raised objections under Article 84 EPC. Opponent 1 has alledged that the term "*control cell line*" contained in the added statement of claim 1 is unspecified. Opponent 2 has noted in the same respect that the features used in claim 3 to define the second cell line have not been introduced in claim 1 to specify the control cell line referred to therein.

7. The Opposition Division is of the opinion that there was no need to add any technical feature in claim 1 with a view to defining more precisely the control cell line, the reason therefor being that a person skilled in the art reading the patent would immediately realize what the features of a cell line to serve the purpose of a control cell line should be. Therefore, **the Division considers that the requirements of Article 84 EPC are met**.

8. Opponents have considered that the present claim request has introduced subject-matter which extends beyond the content of the application as filed. The wording "*control cell line*" referred to thereabove has been regarded as contravening Article 123(2) EPC. Opponent 1 has alleged that the wording "[a protein] *whose presence in a cell evokes a responsive change*" in the preamble of claim 1 would represent an inadmissible generalization of the original disclosure which was limited to active production on the test cell line. Reference was made

to the priority document. Furthermore, Opponent 2 has complained that there is no difference in scope between claims 1 and 3.

9. The Opposition Division regards it as necessary to remind the parties that asessing whether the requirements of Article 123(2) EPC requires a careful comparison of the patent not with the priority document but with the application as originally filed.

9a. The Division is of the opinion that the wording used in present claim 1, account being taken of feature (a) of the characterising part which reads "*providing a cell line which overproduces said protein*", has a clear and unquestionable support in the description. Indeed, the said feature is one of the features which are essential to the performance of the invention as explicitly and constantly expressed throughout the whole description. In this respect, the passage at lines 9-14 on page 4 of the application [see WO 89/07654] in which the inventor has indicated the basis for his invention is of particular relevance. See also the following examplary further passages: page 10, lines 17-35, page 11, lines 25-31, and page 12, lines 10-25 as well as the experimental part of the description.

9b. Moreover, the Division notes that if the scope of claim 1 were to be considered as equivalent to the scope of claim 3 [which has not been considered to contain added subject-matter] as alleged by opponent 3, that would clearly mean that claim 1 does not contain subject-matter which extends beyond the content of the application as originally filed. Furthermore, for the reasons explained at points 2 and 3 the Opposition Division also considers that the expression "control cell line" as used in claim 1 does not introduce subject-matter which extends beyond the content of the application as filed.

9c. In view of the above remarks, **the Opposition Division is of the opinion that the requirements of Article 123(2) EPC are met**.

10. As present claim 1 has been derived from claim 1 as granted by incorporating a limiting additional technical feature and as it would have been obvious to a person skilled in the art that not a "*unique cell*" but "*a cell line*" (a term for which a support can be found in the description of the original application, see for example on

page 4, line 3 of application WO 89/07654) has to be tested when performing the claimed method, **the Opposition Division is of the further opinion that the requirements of Article 123(3) EPC are also met**.

Sufficiency of the disclosure

11. The Opponents have objected that the invention is not described in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art. More particularly, Opponent 3, the only opponent which has based its opposition on that ground for opposition, has stated, while citing document A39 to support its allegation, that the patent does not permit the identification of a direct (in the light of the explanations given by the Proprietor in its letter of 08.04.1998) activator or inhibitor. Relying on the general procedural principle that the burden of proof lays with the opponents and, taken notice of the fact that only theoretical submissions based on a reading of cited documents without the support of any experimental demonstration, **the Opposition Division** considers that it has not been established that the invention is not reproducible and, therefore, **is of the opinion that the requirements of Article 83 EPC are met**.

Validity of the priority

12. The priority validity has been challenged on the assumption that the deposit under the provision of Rule 28(1) EPC of a particular plasmid referred to as pMV7-RP58 was made one day after the priority date with the result that the disclosure of the invention in the priority document would not have been enabling. Moreover, it has been stressed that replacement of the term "*production by a cell*" as found in claim 1 of the priority document by the term "*presence in a cell*" as found in each of claims 1 and 3 of the patent would have extended the claimed subject-matter in such a way that the inventions as defined in the patent and in the pirority document are not the same.

13. Plasmid pMV7-RP58, also denoted pMV7-PKC beta1, is cited on pages 6, 25 and 42 of the application as originally filed (see pages 6, 25 and 52 of application WO 89/07654). This plasmid contains the full-length cDNA sequence of clone RP58 represented on Figure 1A which encodes PKCbeta1. The cells tested in the

experimental part of the description were transfected using said plasmid. The importance of the said plasmid relies on the fact that it contains the said cDNA sequence. On page 42, it is confirmed that the deposit of a culture of *E. coli* bearing it was actually made on 11.02.1988, i.e., one day after the priority date. Whereas it is unambiguous that the said plasmid has been made available to a person skilled in the art according to the required conditions and allow him to reproduce the invention as described in the European application as filed, the question which remains to be assessed is whether the said person would have been in a position to perform the invention on the basis of the priority document without being given the opportunity to be provided with the said plasmid.

14. As noted by the Proprietor at the oral proceedings and indicated in the description of the application as filed (see page 6, line 5 and page 25, line 27 of application WO 89/07654), the PKC cDNA clone RP58 is a full-length clone isolated from a rat brain cDNA library, which corresponds to the clone RP41 as previously reported in document A19 which enjoys a publication date of February 1987. It is therefore considered by the Opposition Division that a person skilled in the art would have been in a position at the priority date using the teaching of document A19 to clone again the clone RP41 and to prepare a plasmid equivalent to plasmid pMV7-RP58 on the basis of the general technology of genetic engineering already well-known at this date. The non-availability of plasmid pMV7-RP58 would not have prevented him to carry out the invention as described in the priority document. A deposit made before the priority date would have only abbreviated his efforts.

15. Even if in the priority document, it is not explictly written that **the presence** as such **of the POI (protein of interest) in the cell** evokes a responsive change in a phenotypic characteristic, there appears to be no doubt that it is implicitly relied on that concept throughout the whole priority document where it has been abundantly explained that the said POI is overproduced in the cells (see for example the passage bridging pages 13 and 14 of the priority document). Clearly, the overproduction of the POI causes it to be present in the cell and its presence in the cell to evoke a responsive change in a phenotypic characteristic as defined in the patent.

16. Therefore, **the Opposition Division is of the opinion that the priority has been validly claimed**. Consequently, neither of documents A26 and A27 belong to the relevant state of the art as defined in Article 54(2) EPC.

Novelty

17. An essential feature of each of the methods of claims 1 and 3 is that the protein with which the substance tested is supposed to interact is such that its presence in a cell evokes a responsive change in a phenotypic characteristic other than the level of said protein in said cell per se. Such protein will be referred to hereafter as a POI (as it has been done in the patent). Moreover, the description clearly indicates that the substance to be tested should interact directly with the POI. The said substance should specifically inhibit or inactivate the POI to be recognized as an inhibitor or an activator and be distinguished from substances which affect cell morphology or growth by other mechanisms in that the substance to be tested will have a greater effect on the test lines than on the control cell lines (see page 3, lines 17-19 of the patent). In other terms, inhibitors and activators of the POI should be distinguished from agents that act upon other cell metabolites to effect a phenotypic change (see page 5, lines 44-46 of the patent).

18. The following documents belonging to the prior art as defined in Article 54(2) EPC have been cited by the parties either at the oral proceedings or only in their written submissions (such documents are noted WS) as being novelty-destroying: A2, A7, A8, A9, A11, A12, A13, A18, A24(WS), A25(WS), A31, A32, A33 (WS), A34, A35, A36, A37, A38 and A39.

19. **Document A2** reports that in examining possible synergistic interactions between tumor promoters and a cloned oncogene the authors have found that the tumor-promoter TPA markedly enhanced the transformation of C3H 10T1/2 mouse fibroblasts when these cells were transfected with the cloned human bladder cancer c-ras^{H} oncogene (see abstract on page 552 together with the low part of the left column of page 553).

19a. There is no indication in document A2 that the substance tested, i.e., TPA, is an inhibitor or an activator of a particular protein whose presence in a cell evokes a

responsive change in a phenotypic characteristic. No such protein (POI) is identified therein.

20. **Document A7** reports that looking for compounds which inhibit the activity of malfunctioning oncogene(s), the authors have tested herbimycin (see Fig. 2 on page 673) for its activity to convert the transformed morphology of Rous sarcoma virus-infected rat kidney cells to the normal morphology.

20a. According to page 674, right column of document A7, the results obtained suggest that herbimycin has no direct effect on *src* kinase but destroys its intracellular environment, resulting in a irreversible alteration of the enzyme leading to loss of catalytic activity. As there is no direct interaction between herbimycin and *src* kinase, herbimycin cannot be regarded as an inhibitor or an activator of the said enzyme. Herbimycin appears to be only an agent that acts upon other cell metabolites to effect a phenotypic change (see, page 5, lines 45-46 of the patent).

21. **In document A8**, it has been demonstrated that 250 nucleotides of DNA contain the sequences necessary for transcriptional regulation of the bovine prolactin gene in rat pituitary cells by three differents agents: steroid hormone inhibition using the synthetic glucorticoid dexamethasone, as well as stimulation by the polypeptides hormones EGF and TRH (see left-hand column of page 12250).

21a. There is no indication in document A8 that any of the substances tested, i.e., dexamethasone, EGF and TRH, interacts with a particular protein as defined in claim 1 as an inhibitor or an activator thereof.

22. **In document A9**, the authors have shown that exposure of *neu*-oncogen-transformed NIH 3T3 cells to monoclonal antibodies with the *neu* gene product, protein p185, results in the rapid and reversible loss of both cell-surface and total cellular p185. Furthermore, the said monoclonal anti-p185 antibody treatment causes neu-transformed NIH 3T3 cells to revert to a nontransformed phenotype.

22a. There appears to be a fundamental difference between the methods of the patent and the experiments reported in document A9: whereas in the methods of the patent what is considered and tested is not simply a variation of the level of the

POI but a variation of its expression, in document A9 the substance tested (the monoclonal antibody) takes away the protein (p185) from the system with no direct effect on its expression.

23. **In document A11**, it has been shown that primary rat embryo fibroblasts containing a *ras*, but not a *myc* concogene, are strongly and specifically stimulated to grow by the tumour promoter TPA.

23a. There is no indication in document A11 that a POI is present in the tested fibroblasts with which TPA would have interacted, the said interaction having resulted in a stimulation to grow. In this respect, what can be acknowledged is that TPA is able to collaborate with a *ras* oncogene to allow focal overgrowth in monolayer culture (see left-hand column of page 703) but the nature of the precise mechanism involved remains to be explained.

24. **In document A12**, it is reported that rat embryo fibroblast cell line 6 was transfected with plasmid pT24, which contains the activated human bladder *c-Ha-ras* oncogene, and the cells were grown continuously in the absence or presence of the tumor promoters TPA or telocidin. The presence of TPA or teleocidin led to a significant increase in the number of morphologically transformed loci, whereas no transformed foci were seen when the said cells were transfected with the normal *c-Ha-ras* oncogene in the absence or presence of TPA, or in cells simply treated with TPA or teleocidin.

24a. There is no indication in document A12 that a POI is present in the tested fibroblats with which TPA would have interacted, the said interaction having resulted in an increase of the number of morphologicall transformed loci. In this respect, what can also be acknowledged is that TPA is able to collaborate with a ras oncogene to allow a phenotypic response but the nature of the precise mechanism involved remains to be explained.

25. **Document A13** reports that with a view to determining the role of TGF-α in the acquisition of a malignant type, a plasmid which directs the constitutive synthesis of human TGF-α was introduced into established nontransformed Fischer rat fibroblasts. Expression and subsequent secretion of TGF-α resulted in a lost of

anchorage dependence. These cells also displayed reduced contact inhibition and were able to form tumors when injected into nude mice. The transformed phenotype in culture could be reversed by anti-TGFα monoclonal antibodies, indicating that TGF-α is secreted and then exerts its effects through receptors on the cell surface (see bottom of the right-hand column of page 304).

25a. It is clear that the addition of monoclonal antibodies resulted in the removal of the protein (TGF-α) which had been secreted in the cells. That means that, as explained above with respect to document A9, the monoclonal antibodies took away the protein from the system with no direct effect on its expression.

26. **Document A18** reports experiments aiming at determining the mechanism of activation of gene transcription by the tumor promoter TPA and explaining the precise role of protein kinase C (PKC) in this mechanism.

26a. The authors came to the suggestion that activation of PKC may not be sufficient to explain all the effects of TPA. In fact, no appropriate inhibitor or activator of PKC could be identified. In this respect (see left-hand column of page 93), it is indicated that the compound bryostatin considered as a potent activator of PKC lacks tumor-promoting activity on mouse skin and does not mimic the effects of TPA on cell differentiation and that the effect of TPA was not blocked by PKC inhibitors. In this respect, it is worth to note the pure speculative value of the statement on page 94 relating to possible assays which could be used to detect potential tumor promoters and related compounds.

27. **Document A24** reports experiments carried out with a view to investigating the selective adavantage, if any, that overexpression of the normal EGF receptor might confer to cells in a model system. It was proved that overexpression of the EGF receptor confers a conditional growth advantage to NIH 3T3 cells.

27a. Nevertheless, the aim of the said experiments was not to test whether a particular substance was an inhibitor or an activator of the EGF receptor, with the results that document A24 failed to identify any possible activator or inhibitor of a POI.

28. **Document A25** relates to a conjugate of M-CSF, which is capable of binding to

the *c-fms* proto-oncogen/M-CSF receptor gene product on certain cancer cells characterized by the over-expression of the said protein, and a cytotoxic agent capable of being transported through the cell membrane and acting in the cytoplasm to destroy the cell.

29a. No method of determining whether a substance is an inhibitor or an activator of a POI is described. What is proposed by document A25 is only the use of a conjugate having first moiety being capable of binding a protein receptor which has been over-produced and is present on the surface of the cell, and second moiety which is a cytotoxic agent capable of entering the cell and subsequently destroying it.

30. **In document A31** it has been shown that human glucocorticoid receptor (hGR) produced in CV-1 cells via transfection of an hGR expression vector functions as a necessary and sufficient factor for the transcriptional activation of the MTV-CAT fusion gene.

30a. It has been alledged that document A31 was disclosing a method as defined in the patent, the dexamethasone being the substance interacting with the POI, namely hGR. Such a method cannot be recognized in the document, at least for the reason that dexamethasone does not intereact with hGR but turns on the transcription of the MTV-CAT fusion gene (see the first full paragraph of the left-hand column of page 647).

31. **Document A32** reports on experiments which have shown that activation of the Xenopus vitellogenin estrogen responsive element (ERE) in various cell lines is dependent upon both the expression of the estrogen receptor (a protein) and the addition of estradiol. The experiments involved transient cotransfection of the estrogen receptor cDNA and the vitellogenin gene-CAT constructs containing the ERE.

31a. It appears that no substance has been tested to determine whether it inhibits or activates a POI. In this respect, it has not been shown that oestradiol was an activator or inhibitor within the meaning of the patent of the estrogen receptor. It has only been shown that oestradiol cooperates with the estrogen receptor to

activate the estrogen responsive element.

32. **Document A33** reports experiments which aimed at using mitogenic monoclonal antibodies against *Thy-1* in concert with transfection of a cloned *Thy-1* gene, first to analyze the precise role of *Thy-1* in T cell activation and then to determine the relationship between anti-*Thy-1*-induced activation, and that produced in response to plant lectins or to stimulation via the T3-Ti complex.

32a. It appears that in document A33 no substance has been tested to determine whether it inhibits or activates a POI in the conditions of the methods of the patent. Whereas it has been shown that the interaction of certain monoclonal antibodies with the T cell differentiation antigen *Thy-1* molecules of murine T lymphocytes leads to cell activation and proliferation, the precise mechanism underlying this process has not been elucidated (see the last paragraph of page 297). It has not been established that the presence of *Thy-1* in a cell overproducing said protein on its own would evoke a responsive change in a phenotypic characteristic said responsive change being influenced by the addition of anti-*Thy-1* monoclonal antibodies.

33. **Document A34** reports that human insulin receptor cDNA was placed into a vector under the control of the simian virus 40 early promoter and its function was tested by transient expression in microinjected Xenopus oocytes and by expression in stably transformed CHO cells. Furthermore, the authors showed that by the use of a monoclonal antibody specific for the human insulin receptor, it is possible to discriminate between endogenous receptors and those produced via the transfected gene.

33a. It appears that no substance has been tested to determine whether it inhibits or activates a POI. In this respect, the aforementioned monoclonal antibody was not tested to determine whether it might inhibit insulin as it was alledged at the oral proceedings. The said antibody had only been used as a discrimination means as indicated above. Moreover, the cell lines used as such were not overproducing a POI. They were able to produce the insulin receptor only upon transfection.

34. **Document A35** reports that to assess the requirement for receptor protein kinase

activity in insulin action, lines of CHO transfectants were developed that expressed equivalent numbers of either normal human insulin receptors or human insulin receptors in which lysine at the ATP-binding site (Lys-1018) has been converted to alanine. Transfectants containing receptors with this substitution expressed processed receptor that bound insulin but lacked protein tyrosine kinase activity.

34a. In figure 4 on page 1844 which shows the stimulation of deoxyglucose uptake when transfected CHO cell lines are tested, a comparison of the upper two curves (transfected CHO expressing mutated receptor and transfected CHO expressing wild type receptor) shows no significant difference, which means that there is no phenotypic changes unless insulin is added. Therefore, the human insulin receptor cannot be regarded as a POI.

35. In the experiments of **document A36**, the aim of the authors was to determine whether the *c-myc* gene product functions as an intracellular mediator of the mitogenic response to platelet-derived growth factor [PDGF] (see page 658, the first sentence of the "Discussion"). Using mouse mammary tumour virus promoter: C-myc recombinant plasmids, 3T3 sublines were constructed in which hydrocortisone was the primary determinant of *myc* mRNA content.

35a. In the said experiments, the c-myc oncoprotein cannot be regarded as a POI. None of the substances used (EGF, PDGF, hydrocortison) have been shown to modulate the activity of the c-myc oncoprotein.

36. **In document A37**, it is reported that focus formation can be induced after transfer of polyoma *plt* and *myc* oncogenes into either rat embryo fibroblasts or established FR3T3 rat cells by subsequent exposure to the tumour promoter 12-O-tetradecanoylphorbol 13-acetate (TPA).

36a. It appears that document A37 does not contain any proof that TPA which is the substance tested actually acts as an inhibitor or an activator of any of the proteins encoded by the said genes. It is worth to note that the said proteins are not mentioned. Nor is their level in the cells measured. Moreover, the phenotypic characteristic in which the presence of such a protein, if regarded as a POI within

the meaning of the patent, would be supposed to evoke a responsive change is not unambiguously determined. In this respect, it has not been established that the said proteins as such would induce the appearance of transformed foci. Furthermore, the last sentence of the last paragraph of page 239 clearly indicates the underlying aim of the authors which was not the identification of a substance being an inhibitor or activator of a POI, but the discovery of new oncogenes.

37. The authors of **document A38** have utilized the cDNA clone of the epidermal growth factor (EGF) receptor. The cDNA has been cloned into a retrovirus expression vector and transfected into NIH/3T3 fibroblast cells. Mutations have been made in the transfected plasmid to study the importance of specific sequences of different receptor domains.

37a. In the reported experiments, it has not been shown that EGF when added to cells overproducing the EGF receptor results in the said cells exhibiting a responsive change in a phenotypic characteristic, which characteristic is associated with the presence of EGF receptor in the cells in the absence of EGF. In this respect, it should be stressed that the receptor mutant having a four amino acid insertion mutation at residue 708 does not cause cells overproducing said mutant to undergo DNA synthesis unless EGF is added, which means that the phenotypic characteristic in which a responsive change would be expected is not exhibited by the cells in the presence of the sole EGF receptor.

38. **Document A39** reports experiments according to which recombinant M2 muscarinic receptors were expressed in cells lacking endogenous receptor to investigate whether a particular receptor subtype can be coupled to multiple effector systems. CHO cells were stably transfected with a vector directing the expression of the porcine atrial M2 muscarinic acetylcholine receptor.

39a. The experiments of document A39 appear not to rely on a cell-based assay as defined in either of claims 1 and 3. In this respect, it has not been established that addition of carbachol would cause the transfected CHO cells to exhibit a responsive change in a phenotypic response evoked in the absence of carbachol by the tested receptor when present in the said cells.

40. In view of the above remarks, the Opposition Division comes to the conclusion that the Opponents have failed to establish that the subject-matter to which is directed either of claims 1 and 3 lacks novelty on the basis of any of documents A2, A7, A8, A9, A11, A12, A13, A18, A24, A25, A31, A32, A33, A34, A35, A36, A37, A38 and A39. It is considered that in none of them all the technical features of the claimed methods are present. Moreover, the point has to be stressed that most of the cited documents deal with particular matters which are far away from experimental protocols especially designed with a view to identifying substances being an inhibitor or activator of a protein, regarded as a POI within the meaning of the patent. This is particularly apparent when considering documents A31 to A39 first cited by Opponent 2 in its letter of 07.10.1999.

41. The Opposition Division concludes that none of the cited documents discloses cell-based or screening systems wherein a cell containing a higher level of a given protein ("the Protein-of-interest ("POI")) is created and/or utilized to determine whether or not a substance is an inhibitor or an activator of the biological activity of the POI by determining alterations in a responsive change in a phenotypic characteristic of said cell to treatment with said substance. Therefore, the subject-matter of each of claims 1 and 3 as well as that of dependent claims 2 and 4 to 23 is regarded as new by the Opposition Division.

42. The same conclusion applies *de facto* to the subject-matter of independent claim 24 as it is directed to the use of a cell line which overproduces a selected protein in a method according to any one of claims 1 to 22. The subject-matter of the said claim is therefore recognised as new.

43. Also in view of the above remarks, the opposition Division is of the opinion that a test kit for carrying the method of any one of claims 1 to 23 which comprises a cell line that overproduces a POI whose presence in a cell evokes a responsive change in a phenotypic characteristic other than the level of said protein in said cell per se is not disclosed in any of the said documents, inclusive of documents A2, A11 and A12. No kit is provided containing a cell line which, once having been shown to be capable of exhibiting said responsive change in a phenotypic characteristic, is then demonstrated to be capable of being used to identify inhibitors or activators of said POI's cellular biological activities.

44. Therefore, **the Opposition Division acknowledges that the claimed subject-matter as a whole is new, in accordance with the requirements of Article 54 EPC.**

Inventive step

45. At the oral proceedings, the opponents have clearly and limitedly expressed the opinion that either document A12 or document A7 should be regarded as the closest prior art document and that in view of a combination of the said documents possibly being also taken account of document A2 the invention would not involve an inventive step.

46. It has been alleged that all the technical features of the method and kit claims are suggested in document A12 and that document A7 discloses a screening method over which the claimed methods do not exhibit any surprising effect.

47. Opponent A3 has submitted that in view of document A7, taken as the most relevant state of the art, the technical problem could be regarded as the provision of a more specific assay than the one of said document, being associated with the drawback that the POI, the *src* kinase, is introduced in the form of a virus that contains additional proteins which causes the described screening assay not to be specific enough. It has been further submitted that since document A7 indicates (see the last paragraph of page 674) a theoretical generic applicability of the method described therein for antitumor agents acting on oncogene function, there would be an incentive to adapt the assay according to document A7 to *ras* oncogene expressing systems as disclosed in document A12.

48. Documents A2, A7 and A12 have already been discussed, see points 15, 15a, 20, 20a, 24 and 24a above. In document A2, there is no indication that the substance tested, the tumor promoter TPA is an inhibitor or an activator of a particular protein whose presence in a cell evokes a responsive change in a phenotypic characteristic. No such protein is identified therein. In document A7, as there is no direct interaction between herbimycin and *src* kinase, herbimycin cannot be regarded as an inhibitor or activator of the said enzyme. In document A12, there is no indication that a POI is present in the tested fibroblasts with which TPA would

have interacted, the said interaction having resulted in an increase of the number of morphologically transformed loci. Therefore, it is considered that none of these references, individually or taken together discloses or suggests a cell-based assy system whereby a chemical substance can be determined to be an activator or an inhibitor of a given target protein and which interacts directly with said target protein. The same conclusion also applies to any other cited prior art document on which the opponents have not relied at the oral proceedings.

49. In view of the above remarks, the Opposition Division considers that any combination of the cited documents would be ineffective at solving the technical problem which can be regarded as the provision of (i) a rapid and easy method for the detection of specific chemical activators and inhibitors of a protein which interact directly with the protein and modulate its cellular activity(ies) in a useful manner and (ii) cells adapted thereto.

50. Therefore, **the Opposition Division** considers that the Opponents have failed to establish that the various claimed aspects of the invention do not involve an inventive step and **concludes that the requirements of Article 56 EPC are met**.

Concluding statement

51. In view of the above remarks, **the Opposition Division** is of the opinion that, taking into consideration the amendments made by the proprietor of the patent during the opposition proceedings, the patent and the invention to which it relates meet the requirements of the Convention, and **decides to maintain the patent on the basis of the claim request filed and amended during the oral proceedings and the description brought into harmony therewith at the same time, under the provisions of Article 102(3) EPC**.

Annex 1

List of documents submitted by the parties during the opposition proceedings

Document	Party/reference	Bibliographic data
A1	O3/D4; PR/D1	*Cancer Research*, 40, 1980, 4709-16
A2	O1/D2; O2/D14; PR/D2	*Science*, 226, 1984, 552-5
A3	O1/D3; PR/D3	*Cancer Cells*, 1, 1984, 229-37
A4	O1/D6; PR/D4	*Cancer Cells*, 1, 1984, 239-44
A5	O1/D7; PR/D5	*Cancer Cells*, 1, 1984, 263-9
A6	O3/D3; PR/D6	*Nature*, 308, 1984, 693-8
A7	O1/D5; PR/D7	*Jpn. J. Cancer Res.*, 76, 1985, 672-5
A8	O1/D10; PR/D8	*J. Biol. Chem.*, 260, 1985, 12446-51
A9	O2/D3; PR/D9	*Cell*, 41, 1985, 695-706
A10	O2/D9; PR/D10	*Chemotherapy*, 31, 1985, 440-50
A11	O2/D13; PR/D11	*Nature*, 318, 1985, 472-5
A12	O1/D1; O3/D2; PR/D12	*Mol. Cell. Biol.*, 6, 1986, 1943-50
A13	O2/D1; PR/D13	*Cell*, 46, 1986, 301-9
A14	O2/D7; PR/D14	*Cell*, 46, 1986, 491-502
A15	O2/D15; PR/D15	*Science*, 233, 1986, 478-81
A16	O3/D5; PR/D16	J.E. Darnell, Mol. Cell. Biol., Scientific American Books Inc. (1986)
A17	O1/D4; O2/D6, PR/D17	*Mol. Cell. Biol.*, 7, 1987, 2821-9
A18	O1/D8; PR/D18	*Environmental Health Perspectives*, 76, 1987, 89-95

Document	Party/reference	Bibliographic data
A19	O1/D9; O2/D8; PR/D19	*Proc. Natl. Acad. Sci. USA*, 84, 1987, 1065-9
A20	O2/D5; PR/D20	*Cancer Res.*, 39, 1987, 173-83
A21	O2/D10; PR/D21	*Science*, 236, 1987, 305-8
A22	O2/D11; PR/D22	*Cancer Cells*, 3, 1987, 359-63
A23	O2/D12; PR/D23	*Proc. Natl. Acad. Sci. USA*, 84, 1987, 8903-6
A24	O3/D1; PR/D24	*Cell*, 51, 1987, 1063-70
A25	O2/D2; PR/D25	WO 89/03687
A26	O2/D4; PR/D26	*Adv. in Exp. Med. Biol.*, 234, 1988, 127-140
A27	O1/D11; PR/D27	*Cell*, 52, 1988, 343-54
A28	PR/D28	Alberts, Molecular Biology of the Cell, 3rd ed. (1984), Garland Pub., NY USA, 1264-5
A29	PR/D29	Chapter 2 of "The Pharmacological Basis of Therapeutics", Goodman & Gilman (9th Ed. 1996)
A30	PR/D30	Declaration of Dr. Housey dated 06.04.1998, together with four exhibits numbered 1 to 4
A31	O2/A31	*Cell*, 46, 1986, 645-52
A32	O2/A32	*Nucleic Acids Research*, 14(23), 1986, 9329-37
A33	O2/A33	*Journal of Experimental Medicine* 163, 1986, 285-300
A34	O2/A34	*Proc. Natl. Acad. Sci. USA* 82, 1985, 8014-8
A35	O2/A35	*The Journal of Biological Chemistry* 262(4), 1987, 1842-7

Document	Party/reference	Bibliographic data
A36	O2/A36	*Nature*, 310, 1984, 655-60
A37	O2/A37	*Nature*, 314, 1985, 277-9
A38	O2/A38	*The EMBO Journal*. 5(9), 1986, 2179-90
A39	O2/A39	*Science*, 238, 1987, 672-5
A40	O1/A31	*Biochemical and Biophysical Research Communications*, 145(2), 1987, 782-8
A41	O1/A32	*Molecular and Cellular Biology*, 3(6), 1983, 1123-32
A42	O3/BI1	*J. Physiol.*, 80, 1985, 229-32
A43	O3/BI2	*Nature*, 327, 1987, 623-5

Annex 2

Claim 1 as granted:

A method of determining whether a substance is an inhibitor or an activator of a protein whose presence in a cell evokes a responsive change in a phenotypic characteristic other than the level of said protein in said cell per se, which comprises:

a) providing a cell which overproduces said protein and exhibits said phenotypic response to the protein;

b) incubating said cell with said substance; and

c) determining whether said cell exhibits a responsive change in a phenotypic characteristic.

Claim 1 filed with the letter 23.04.1998:

A method of determining whether a substance is an inhibitor or an activator of a protein whose presence in a cell evokes a responsive change in a phenotypic characteristic other than the level of said protein in said cell per se, which comprises:

a) providing a cell which overproduces said protein and exhibits said phenotypic response to the protein;

b) incubating said cell with said substance; and

*c) determining whether said cell exhibits a responsive change in **said** phenotypic characteristic.*

(said claim differs from claim 1 as granted in that the term ***said*** has been introduced thereinto to take the place of an article "a")

< comparing the phenotypic response of said cell line of (a) to the substance with the phenotypic response of a control cell line to the substance to >

Jae 9/11/95

Main ~~Auxiliary~~ Request

CLAIMS

1. A method of determining whether a substance is an inhibitor or an activator of a protein whose presence in a cell evokes a responsive change in a phenotypic characteristic other than the level of said protein in said cell per se, which comprises:
   (a) providing a cell line which overproduces said protein and exhibits said phenotypic response to the protein;
   (b) incubating said cell line with said substance; and
   (c) determining whether said cell line of (a) exhibits a responsive change in said phenotypic response.

2. The method of claim 1, wherein said responsive change is a graded cellular response to said substance.

3. A method of determining whether a substance is an inhibitor or activator of a protein whose presence in a cell evokes a responsive change in a phenotypic characteristic other than the level of said protein in said cell per se, which comprises:
   (a) providing a first cell line which overproduces said protein and exhibits said phenotypic response to the protein;
   (b) providing a second cell line which produces the protein at a lower level than the first cell line, or does not produce the protein at all, and which exhibits said phenotypic response to the protein to a lesser degree or not at all;
   (c) incubating the first and second cell line with the substance; and
   (d) comparing the phenotypic response of the first cell line to the substance with the phenotypic response of the second cell line to the substance.

4. The method of any one of claims 1 to 3, wherein the response is one observable with the naked eye.

5. The method of any one of claims 1 to 4, wherein the response is a change in a cultural or morphological characteristic of the cell.

6. The method of claim 5, wherein the response is a change in the differentiation state of the cell.

7. The method of any one of claims 1 to 4, wherein the response is a change in the ability of the cell line to grow in an anchorage-independent fashion.

8. The method of any one of claims 1 to 4, wherein the response is a change in the ability of the cell line to grow in soft agar.

9. The method of any one of claims 1 to 4, wherein the response is a change in foci formation in cell culture.

10. The method of any one of claims 1 to 4, wherein the response is a change in the ability of the cell to take up a selected stain.

11. The method of any one of claims 1 to 10 in which the protein is an enzyme.

12. The method of claim 11, wherein increased activity of the enzyme is correlated with increased tumorigenesis.

13. The method of claim 12 in which the enzyme is a protein kinase C enzyme or a fragment, domain or subunit of a receptor which has protein kinase C activity.

14. The method of claim 12, wherein the enzyme is ornithine decarboxylase.

15. The method of any one of claims 1 to 10 in which the protein is the expression product of an oncogene.

16. The method of any one of claims 1 to 10, wherein the response is a change in

an antigenic characteristic of the cell.

17. The method of any one of claims 1 to 16 in which the substance is a suspected inhibitor of the biological activity of the protein.

18. The method of any one of claims 1 to 16 in which the substance is a suspected activator of the biological activity of the protein.

19. The method of any one of claims 1 to 18, wherein said first cell line is obtained by introducing a gene encoding the protein of interest into a host cell, said gene being under the control of a promoter functional in the host cell, whereby said gene is expressed.

20. The method of claim 19, wherein the gene is introduced into the host cell by means of a first genetic vector into which the gene has been inserted, and said second cell is obtained by introducing into a similar host cell a second genetic vector essentially identical to the first genetic vector except that it does not bear said gene insert.

21. The method of claim 19 or 20, wherein the gene is introduced into the host cell by means of a retroviral vector.

22. The method of any one of claims 19 to 21 in which the host cell essentially does not produce the protein.

23. The method of any one of claims 19 to 22 in which the host cell is a rat-6 fibroblast cell line.

24. Use of a cell which overproduces a selected protein in a method according to any one of claims 1 to 22 for the determination of whether a substance is an inhibitor or an activator of said selected protein.

25. A test kit for carrying out the method of any one of claims 1 to 23, which comprises:

(a) a first cell which overproduces the protein and exhibits said phenotypic response thereto; optionally in combination with

(b) a second cell which produces the protein at a lower level than the first cell, or does not produce the protein at all, and which exhibits said phenotypic response to the protein to a lesser degree or not at all.

26. The test kit of claim 25, wherein the level of production of the protein in the first cell is at least five times the level of production of the protein in the second cell.